Respiratory syncytial virus-related disease burden in young children

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Respiratory syncytial virus-related disease burden in young children

Respiratoir syncytieel virus-gerelateerde ziektelast in jonge kinderen
(met een samenvatting in het Nederlands)

Proefschrift

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Nienke Marije Scheltema
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Promotoren: Prof. dr. L.J. Bont
Prof. dr. C.K. van der Ent

Contents

1

Introduction

Respiratory syncytial virus infection

Respiratory syncytial virus (RSV) is a Paramyxovirus with two known virus strains, RSV A and RSV B. Practically all children experience at least one RSV infection during the first two years of life.[1] RSV infects the epithelium of the respiratory tract and the majority of infected children develop upper respiratory tract symptoms, such as rhinorrhoea, nasal congestion, sore throat, cough, mild fever or otitis media.[2,3] When RSV leads to lower respiratory tract infection, typically bronchiolitis, symptoms of cough, wheezing, fast and laboured breathing, or hypoxia develop. Hospitalisation is required in 10% of children with such RSV-related lower respiratory tract infection, making RSV infection a leading cause of hospitalisation during infancy.[4] RSV is the causative pathogen in 19-27% of children younger than 12 months who are hospitalised for acute (lower) respiratory tract infection.[2,5–7] This corresponds to an estimated global RSV-related hospitalisation rate in children younger than 12 months of 19.19 (95% CrI 15.04-24.48) per 1000 children per year.[8] The RSV Global Epidemiology Network (GEN) has estimated the global number of RSV infections in young children (younger than five years) based on published and unpublished data around the world. They estimated that globally, 33.1 million (uncertainty rage 21.6-50.3) episodes of RSV-related acute lower respiratory tract infection occurred in children younger than five years in 2015 and 3.2 million (uncertainty range 2.7-3.8) of these episodes required hospital admission.[4]

In the Netherlands RSV knows yearly seasonality with peak incidence in December and January. The season typically starts between September and December and lasts five to six months.[9] The same pattern is seen in other countries in the Northern Hemisphere, while countries in the Southern Hemisphere like Argentina and Brazil show peak RSV incidence between April and June. Year-round or prolonged (10 months) RSV activity is seen in countries with subtropical climates like Taiwan.[9,10]
The main source of infection is the infant's household; especially older school-attending siblings introduce RSV into households.[11] The presence of siblings and day-care attendance both increase the risk for RSV-related hospitalisation.[12–14] Other risk factors for RSV hospitalisation are age younger than six months, prematurity, birth close to the RSV season, smoke exposure, lack of breastfeeding, family history of atopy and male sex.[2,12,14,15] Although the presence of risk factors increases the risk for hospitalisation, in absolute numbers the majority of infants (79-85%) hospitalised for RSV are those born term and healthy.[5,16]

Unfortunately, there is no treatment for RSV bronchiolitis except supportive care by use of supplemental oxygen, nasogastric or intravenous fluids and ventilatory

support.[17] Evidence is inconclusive for the role of systemic corticosteroids, nebulised hypertonic saline, nebulised epinephrine, antiviral therapy or antibiotics in treatment of bronchiolitis.[17] As a result, it is currently not possible to shorten clinical disease course or to prevent clinical deterioration to life-threatening disease requiring ventilatory support and paediatric care unit admission.

RSV-related childhood mortality

In high-income countries RSV-related mortality is generally infrequent with an in-hospital case fatality rate of <1%.[4,18,19] RSV-related mortality has, however, been put high on the global child health agenda after two important publications described the global RSV-related mortality burden in children younger than five years in 2010 and 2012.[20,21] In these publications the RSV GEN and Global Burden of Disease (GBD) working groups estimated the global number of RSV-related child deaths and the contribution of RSV to lower respiratory infection-related mortality. The RSV GEN working group estimated 66 000 to 199 000 RSV-related deaths in children younger than five years in 2005.[20] The GBD working group estimated that RSV was the most frequently identified pathogen for lower respiratory infection-related mortality during infancy and that 234 000 children younger than five years had died with RSV in 2010.[21] Both the RSV GEN and GBD working groups have updated their RSV-related mortality estimates for 2015. The RSV GEN working group estimated 59 600 (uncertainty range 48 000-74 500) RSV-related in-hospital deaths in children younger than five years in 2015.[4] The GBD working group estimated RSV to be the causative pathogen in 5.2% (95% UI 2.9-8.6%) of lower respiratory infection-related deaths in children younger than five years corresponding to a total number of 36 400 (95% UI 20 400-61 500) RSV-associated deaths in 2015.[22]

The global in-hospital case fatality rate of RSV-associated lower respiratory tract infection is approximately 2% and most RSV-associated deaths occur in lower income regions.[4,20] Global RSV-related mortality has therefore received particular attention from global child health stakeholders such as the World Health Organisation. Despite the global attention for RSV-related mortality, little is known about the clinical characteristics of children who die with RSV, especially in low-income countries. RSV-related mortality has been sporadically described in studies done in high-income or middle-income countries with small patient numbers.[23–30] The largest case series of 35 RSV-related deaths was done at an intensive care unit in the United Kingdom.[23] As a result of the paucity of clinical data, the age distribution of children who die with RSV is still unknown, although children are likely to be aged younger than 12 months.[28,31,32] The age distribution of RSV-related deaths is important for understanding the potential of

RSV preventive interventions in young children and for defining the preferred prevention strategy.

Risk factors for RSV-related death, other than age, influence the preferred target population for RSV prevention as well. A review of the literature was performed to identify risk factors for global RSV-related death in children younger than five years. Six databases (Medline, Embase, Global Health, Global Health 1973, Cinahl Plus and Lilacs) were searched for studies published between January 1995 and March 2016 that described risk factors for RSV-related death. The search identified more than 10 000 published studies of which approximately 40 were eligible for review. Only studies in which at least 60% of the study population had laboratory-confirmed RSV infection and in which 90% of RSV infections had been community-acquired were included for review. Studies in which the study population consisted of children who had received chemotherapy, bone marrow transplant or organ transplant were excluded. Studies that reported on risk factors for severe disease course after hospitalisation were also excluded as this review aimed to identify predisposing conditions associated with fatal RSV infection only.

Fewer than ten studies reported significant risk factors for RSV-related death and none of the studies were done in low-income countries[23,24,29,31,33–36], reflecting the gap in knowledge about global RSV-related mortality. Therefore, studies were also included for review if they described risk factors for paediatric care unit admission or use of mechanical ventilation as a proxy for mortality in settings without such healthcare facilities. Again there were no data available for risk factors in children from low-income countries. Furthermore, none of the included studies described socioeconomic risk factors related to life-threatening RSV infection.

The following risk factors for life-threatening RSV infection including death were reported most frequently: congenital heart disease, prematurity, chronic lung disease, age younger than six months at admission and neurological disease.[23,30,31,33,34,36–44] Less frequently reported risk factors for life-threatening RSV infection including death were: failure to thrive[45], HIV infection[29,35] and Down's syndrome.[24] However, study populations, case definitions and risk factor calculations varied substantially between the reviewed studies limiting comparability of reported results.

RSV prevention

To date, there is no vaccine available to prevent RSV infection. Immunity after natural RSV infection is partial and transient, making re-infection with RSV common.[1,46] In general, primary RSV infection causes most severe disease in children, whereas secondary

and following RSV infections cause milder clinical disease. RSV prevention strategies therefore focus on protection of populations that are at increased risk for severe RSV disease, either via direct or indirect prevention of infection.

The two major surface proteins of RSV, the fusion F-protein and highly glycosylated G-protein, are the major targets of the natural antibody response and thus of immunisation strategies.[47] Passive immunisation with palivizumab, a humanised monoclonal antibody that targets the RSV F-protein, is the only form of RSV prevention currently available. However, due to its cost palivizumab is only indicated in certain high-risk infant populations from high-income countries. Infants with severe prematurity, concomitant chronic lung disease or hemodynamically significant congenital heart disease are recommended to receive palivizumab during the RSV season of their first year of life.[48] Palivizumab has a half-life of 28 days and is therefore administered once a month during the RSV season.[49] As a result, it is desirable to develop alternatives for RSV prevention that are less expensive, easier to administrate and that could become more widely available.

Several other preventive interventions are currently under development.[50,51] These interventions could be categorised into the following immunisation strategies: active immunisation through vaccines, passive immunisation of infants through maternal vaccination, passive immunisation through immunoglobulins (like palivizumab) and antiviral treatment.[51] Several of these strategies are in phase-2 or phase-3 of clinical development (Figure 1).
Due to immaturity of the immune system in neonates, paediatric vaccination is not possible directly after birth but aimed at an age of approximately two to three months. Vaccination of pregnant women is therefore a promising alternative if the youngest infants are targeted to be protected. Maternal vaccination generates an immune response in pregnant women who then transfer the vaccine-induced IgG antibodies via the placenta to the foetus. Thereby, the foetus will be born with an increased level of protective antibodies to provide protection against infection directly after birth (Figure 2). However, due to waning of maternally-acquired protective antibodies after birth, protection against infection will only be temporary. Maternal vaccination against influenza and pertussis has been demonstrated to be safe and effective and to provide protection during the first two to three months of life.[52–55] Knowledge of the peak age at which an infection occurs is therefore crucial for the expected protection by a maternal vaccine. Maternal vaccination against RSV is in phase-3 of clinical development[50,56], but the expected effects of maternal vaccination on prevention of RSV infection and reduction of the RSV disease burden are still unknown.

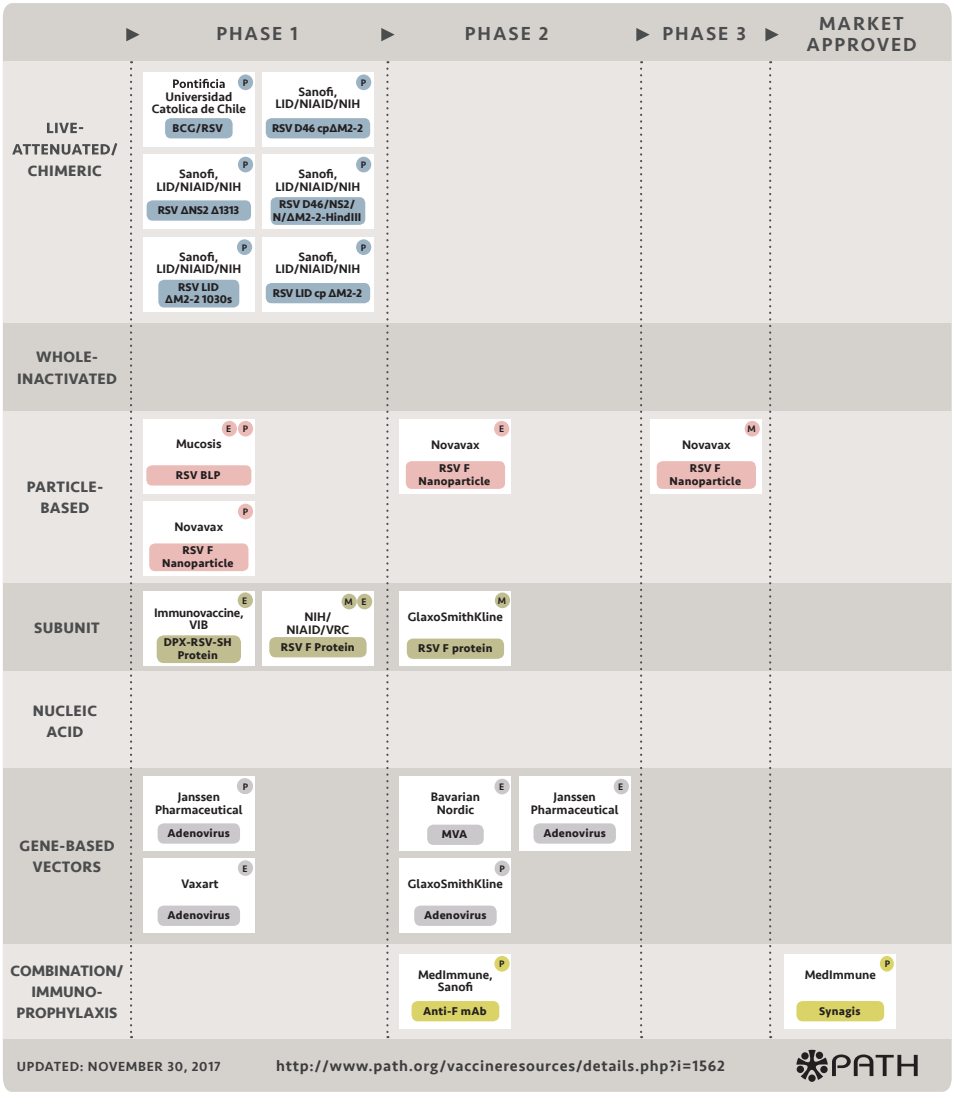

Figure 1. Overview of RSV preventive interventions that are currently in phase-1 of clinical development or beyond

Target populations are P = paediatric, M = maternal, and E = elderly. Adapted from RSV Vaccine and mAb Snapshot published online by PATH on November 30th 2017.[50]

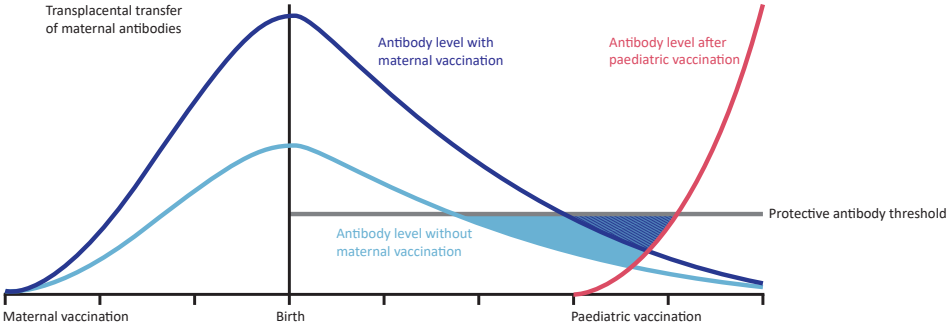

Figure 2. Principles of maternal vaccination and antibody dynamics in mother and child
Maternally-acquired protective antibody levels decrease after birth and once levels are below the protective threshold, the child is no longer protected against infection (blue shaded areas). Maternal vaccination shortens the unprotected period (dark blue shaded area) by increasing the protective antibody level a child is born with. Adapted from Van der Maas et al. Ned Tijdschr Geneeskd. 2016.[57]

RSV and asthma

RSV-related lower respiratory tract infection during infancy has been associated with long-term respiratory morbidity, namely wheeze and asthma. Evidence for this association comes from observational cohort studies that followed children hospitalised for RSV infection early in life and found that up to 50% of children were diagnosed with asthma in later childhood.[58–64] A meta-analysis of cohort studies estimated an overall odds ratio of 3.84 for asthma in children hospitalised for RSV infection early in life.[65] The meta-analysis also showed that the association between RSV hospitalisation and asthma declined with age and that quality of the included studies was generally poor.[65] In addition, observational cohort studies are not designed to provide evidence for causality. The question remains whether RSV infection early in life increases susceptibility to asthma via airway damage, lung function impairment or altered immune response, or whether children with severe RSV infection have pre-existing vulnerability to both severe RSV infection and asthma development (Figure 3). RSV infection requiring hospitalisation could thus be a cause of asthma or an indicator of increased risk for asthma.[66]

In order to study the causal link between RSV infection during infancy and subsequent wheeze and asthma development, several trials have been performed. In the MAKI trial (a multicentre, double-blind, randomised, placebo-controlled trial) otherwise healthy preterm infants were randomised to be protected against RSV infection by receiving the monoclonal antibody palivizumab or to receive placebo during the RSV season of

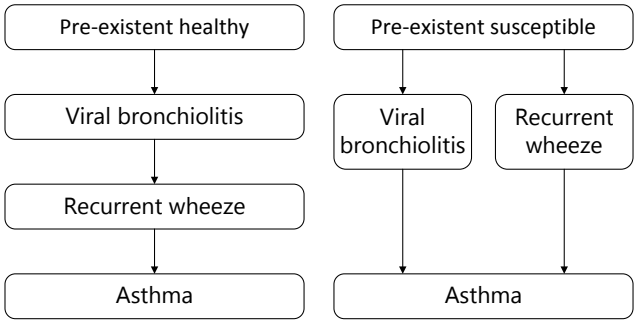

Figure 3. Serial and parallel hypothesis for the relationship between RSV bronchiolitis and development of wheeze and asthma

their first year of life.[67] RSV prevention reduced the total number of days with wheezing symptoms in the first year of life by 61%.[67] A randomised placebo-controlled trial done in healthy term infants in the USA studied the effect of RSV prevention by motavizumab on medically-attended wheezing up to age three years and found no effect.[68] The only evidence for the effect of RSV prevention on wheeze and asthma up to age six years comes from an observational case-control study in otherwise healthy preterm children from Japan.[62] Children who had received palivizumab for RSV prophylaxis had reduced incidence of recurrent wheezing, but not of atopic asthma at age six years.[62] No randomised clinical trial has assessed the causal link between RSV prevention during infancy and subsequent asthma development or lung function impairment.

Objectives and outline of this thesis

In this thesis some knowledge gaps regarding the RSV disease burden in young children are addressed.

The first aim of this thesis is to study global RSV-related childhood mortality in order to define the clinical and socioeconomic characteristics of children who die with RSV infection. Available global RSV disease burden estimates have described the importance of RSV as cause of lower respiratory infection-related mortality in young children, but individual patient data for RSV-related mortality are scarce, especially from low-income regions. Better understanding of the clinical profile of children who die with RSV infection, including age at death, is required to target future RSV preventive interventions with the aim to reduce global childhood mortality. In **chapter 2** individual patient data of children who died with RSV in hospitals across the world are studied to describe the age distribution and most important clinical characteristics in these children.

The second aim is to quantify the potential effect of maternal vaccination on global RSV-related childhood mortality. Maternal vaccination is one of the RSV immunisation strategies that are currently under development and is considered a promising strategy to provide protection against RSV infection during the first months of life. The expected effect of maternal vaccination on RSV prevention in children is unknown. In **chapter 3** factors that influence maternal vaccine efficacy are evaluated by use of a newly developed mathematical model. Using individual patient data in this mathematical model the potential effect of maternal vaccination on prevention of life-threatening and fatal RSV infection in young children is predicted.

Third, this thesis aims to provide conclusive evidence for the presence or absence of a causal relationship between RSV infection and asthma development. To date, no randomised, placebo-controlled clinical trial has assessed the effect of RSV prevention during infancy on asthma and lung function during childhood. Since asthma is one of the most common chronic diseases globally it is important to evaluate potential causative pathways in order to attempt to prevent asthma development, at least to some extent. Prevention of asthma development as a long-term effect of RSV prevention further influences the health economic impact of RSV prevention. **Chapter 4** describes the effect of RSV prevention in otherwise healthy late preterm infants on asthma and lung function by continued follow-up of the MAKI trial study participants until age six years.

A general discussion of the main findings of this thesis can be found in **chapter 5** and a summary in Dutch in **chapter 6**.

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Global respiratory syncytial virus-associated mortality in young children (RSV GOLD): a retrospective case series

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Abstract

Background

Respiratory syncytial virus (RSV) infection is an important cause of pneumonia mortality in young children. However, clinical data for fatal RSV infection are scarce. We aimed to identify clinical and socioeconomic characteristics of children aged younger than 5 years with RSV-related mortality using individual patient data.

Methods

In this retrospective case series, we developed an online questionnaire to obtain individual patient data for clinical and socioeconomic characteristics of children aged younger than 5 years who died with community-acquired RSV infection between Jan 1, 1995, and Oct 31, 2015, through leading research groups for child pneumonia identified through a comprehensive literature search and existing research networks. For the literature search, we searched PubMed for articles published up to Feb 3, 2015, using the key terms "RSV", "respiratory syncytial virus", or "respiratory syncytial viral" combined with "mortality", "fatality", "death", "died", "deaths", or "CFR" for articles published in English. We invited researchers and clinicians identified to participate between Nov 1, 2014, and Oct 31, 2015. We calculated descriptive statistics for all variables.

Findings

We studied 358 children with RSV-related in-hospital death from 23 countries across the world, with data contributed from 31 research groups. 117 (33%) children were from low-income or lower middle-income countries, 77 (22%) were from upper middle-income countries, and 164 (46%) were from high-income countries. 190 (53%) were male. Data for comorbidities were missing for some children in low-income and middle-income countries. Available data showed that comorbidities were present in at least 33 (28%) children from low-income or lower middle-income countries, 36 (47%) from upper middle-income countries, and 114 (70%) from high-income countries. Median age for RSV-related deaths was 5.0 months (IQR 2.3-11.0) in low-income or lower middle-income countries, 4.0 months (2.0-10.0) in upper middle-income countries, and 7.0 months (3.6-16.8) in high-income countries.

Interpretation

This study is the first large case series of children who died with community-acquired RSV infection. A substantial proportion of children with RSV-related death had comorbidities. Our results show that perinatal immunisation strategies for children aged younger than

6 months could have a substantial impact on RSV-related child mortality in low-income and middle-income countries.

Research in context

Evidence before this study

Respiratory syncytial virus (RSV) infection is a leading cause of global acute lower respiratory tract infection in young children. It was associated with 48 000-74 500 in-hospital deaths in children aged younger than 5 years in 2015, with 99% of these deaths occurring in developing countries. However, individual patient data for RSV-related deaths are scarce. We searched PubMed for articles published in English up to July 7, 2017, using search terms related to RSV infection, pneumonia, and childhood mortality. We found case series of RSV-related child deaths that reported 35 cases or fewer.

Added value of this study

We did, to our knowledge, the first case series of children who died with RSV infection to define clinical and socioeconomic characteristics of RSV-related mortality. We searched the literature using PubMed for "RSV", "respiratory syncytial virus", or "respiratory syncytial viral" combined with "mortality", "fatality", "death", "died", "deaths", or "CFR" for articles published up to Feb 3, 2015, in English, to identify research groups with relevant cases and obtained additional cases through existing research networks. We report on 358 in-hospital deaths with laboratory-confirmed RSV infection from 23 countries across the world. A substantial proportion of in-hospital RSV-related deaths occurred in children with pre-existing comorbidities. Most children in low-income and middle-income countries were aged younger than 6 months at the time of death.

Implications of all the available evidence

This study is the first case series of children who died with RSV infection in hospital, giving insight into the clinical and socioeconomic background of children with RSV-related death. Young age at death supports the concept that maternal vaccination against RSV infection could be an effective strategy to prevent RSV-related childhood mortality.

Introduction

Respiratory syncytial virus (RSV) infection is the primary pathogen identified in children with acute lower respiratory tract infection during the first year of life.[1–4] RSV-related acute lower respiratory tract infection is an important cause of death in young children (aged younger than 5 years); approximately 48 000-74 500 children in this age group died in hospital with the condition in 2015.[5] About 99% of RSV-related childhood mortality occurs in developing countries.[5] Although RSV-related mortality in children poses an important global health problem, clinical data for global RSV-related mortality are scarce. Data suggest that most RSV-related childhood mortality occurs during the first year of life.[6–8] Although the case fatality rate is highest in children with underlying conditions, such as congenital heart disease, chronic lung disease, Down's syndrome, or premature birth,[7–13] most cases of life-threatening RSV infection occur among previously healthy children.[14–16] This finding suggests that in settings without intensive care facilities, otherwise healthy children could also be at risk of dying from RSV infection. A study from Argentina[16] reported that poor access to intensive care was associated with RSV-related death.

RSV-related mortality in young children has primarily been described sporadically in studies[10,11,14,17] from intensive care units in high-income or middle-income countries.

To date, the largest case series[11] of 35 RSV-related deaths was reported from an intensive care unit in the UK, with numbers smaller than 35 having been reported in other studies.[2,16,18,19] In 2015, the WHO's Product Development for Vaccines Advisory Committee identified RSV as "a pathogen for which there is major vaccine pipeline activity", with a vaccine likely to be available in the next 5-10 years.[20]

Two broad approaches to RSV immunisation are being considered in young children: maternal immunisation for children aged younger than 6 months and paediatric vaccines for children aged older than 6 months. A good understanding of the age distribution of RSV-related deaths is likely to assist in development of an evidence base to inform vaccine policy, particularly in low-income and middle-income settings.[21]

Previous reports of RSV-related mortality in young children described studies done in one centre, region, or country. None of them were large enough to draw robust conclusions on the clinical and socioeconomic profile of children who die with community-acquired RSV infection globally. To gain insight into the clinical characteristics of RSV-related mortality in young children, we initiated the RSV Global Online Mortality Database (RSV GOLD) study with the aim to gather available retrospective data for fatal community-acquired RSV infections across the world.

Methods

Study design and patients

RSV GOLD is a global study that retrospectively analysed individual data for children aged 0-59 months who died with community-acquired RSV infection between Jan 1, 1995, and Oct 31, 2015. We identified research groups through a comprehensive literature search on PubMed (for articles published up to Feb 3, 2015) using the key terms "RSV", "respiratory syncytial virus", or "respiratory syncytial viral" combined with "mortality", "fatality", "death", "died", "deaths", or "CFR". We limited the search to papers written in English with the abstract and full text available. We invited authors of scientific, peer-reviewed papers reporting RSV-related mortality in children to collaborate. Additionally, we obtained unpublished individual patient data from researchers and clinicians who we identified via research networks. We invited researchers and clinicians to participate in this study between Nov 1, 2014, and Oct 31, 2015. We included data for children with laboratory-confirmed RSV infection and excluded cases of nosocomial or post-stem cell transplantation RSV infection. Since this study only used anonymised secondary data, the institutional research board of the University Medical Centre Utrecht waived the requirement for parental informed consent.

Procedures

We collected data using an online questionnaire designed by a group of investigators (NMS, LJB, HN, FPP, BDG, and DEG). The questionnaire aimed to collect information about clinical and socioeconomic characteristics of children with RSV-related mortality. Characteristics of interest were based on risk factors for RSV-related and pneumonia-related mortality as described in the literature and on expert opinion (appendix).

We carefully validated patient information by direct communication with participating researchers for data entry errors, missing variables, and conflicting data. We deemed age at death within 0-59 months, laboratory-confirmed RSV diagnosis, and community-acquired RSV infection minimum essential data for inclusion. After assessment of data quality, we excluded children if nosocomial infection could not be ruled out on the basis of available clinical information or if death occurred before 1995. We sent additional questions about admission to paediatric intensive care units and availability of mechanical ventilation to the participating researcher if this information could not be extracted from the shared data.

We defined the following patient populations: children with comorbidities, healthy term children, and preterm children without other comorbidities (healthy preterm children). Children with comorbidities had at least one underlying disease, such as congenital heart disease, a genetic or chromosomal disorder, HIV infection, or active tuberculosis.

Healthy term children were born without comorbidities at 37 weeks' gestational age or later and healthy preterm children were born without comorbidities at earlier than 37 weeks' gestational age. When data for comorbidities or prematurity were not recorded, we assumed that the children were healthy term. We categorised countries as high income, upper middle income, lower middle income, or low income on the basis of the World Bank classifications for 2016.[22] We considered Hong Kong and Taiwan high-income countries in line with the World Bank classification. The composite variable neurological disease consisted of neurodevelopmental, neuromuscular, and other neurological disease, such as epilepsy or encephalopathy.

Statistical analysis

We calculated descriptive statistics for all variables. We provide frequencies and proportions for categorical variables. We report the summary results for continuous variables as medians with IQRs. We analysed data by World Bank income regions. Additionally, we analysed data separately for children with comorbidities, healthy term children, and healthy preterm children. Using WHO child growth standards,[23] we calculated Z scores for weight for age, height for age, and weight for height. We did not correct weight for age for gestational age.

To rule out bias, we did sensitivity analyses excluding cases with missing data for prematurity or comorbidities to test our assumption that children with missing data were healthy term. Similarly, we analysed to what extent our results were sensitive to the contribution of a large number of cases from Argentina and Kenya by doing analysis without these countries. We tested meaningful subgroup differences for significance with Kruskal-Wallis or χ^2 statistical tests. We did further paired testing between subgroups using Mann-Whitney U or χ^2 tests with Bonferroni correction for multiple testing. We did not test subgroup differences of results presented in the appendix for significance. We did all statistical analyses with SPSS statistical software (version 22.0).

Results

The literature search resulted in 1154 publications (Figure 1). From these publications, we identified 85 research groups, which we complemented with research groups identified through existing research networks, particularly through the RSV Global Epidemiology Network.[5] We obtained data for 451 children with RSV-related mortality from the 31 research groups that shared data. We obtained both published and unpublished cases. Using original publications to contact research groups, we estimated that we could have missed a maximum of 140 cases from the published literature as most researchers who did not respond to our invitation to participate had reported solitary

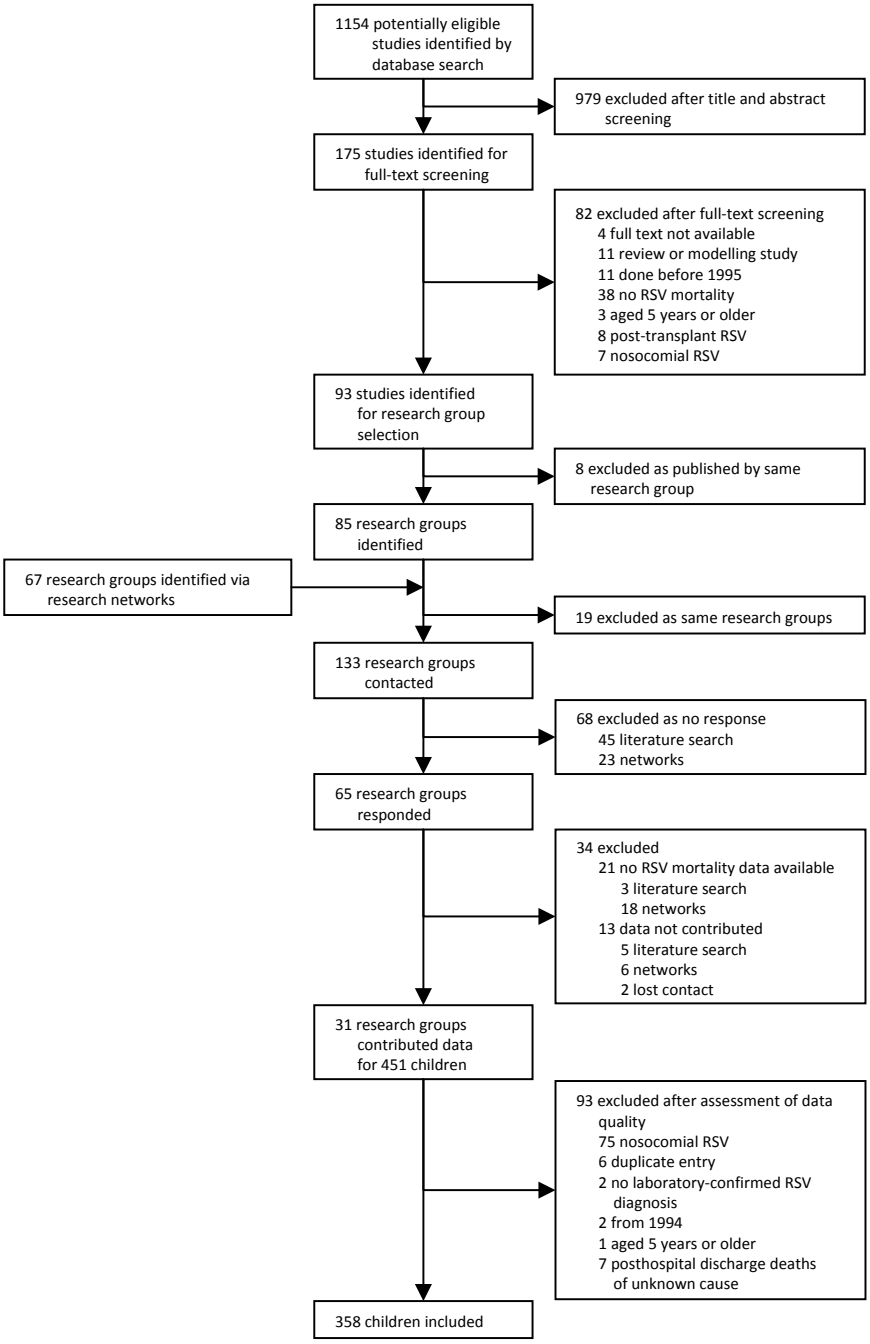

Figure 1. Study selection
RSV = respiratory syncytial virus.

cases of RSV-related mortality: in 28 publications, the number of potential RSV-related deaths was mentioned and in 15 (54%) of those, one or two RSV-related deaths were described. Many non-responders were research groups from high-income countries (41 [60%] of 68) and had published their results between 2010 and 2015 (21 [47%] of 45). Publications used to identify research groups that contributed data are shown in the appendix. After initial assessment of the 451 children, we excluded 93 (21%). Most exclusions were associated with nosocomial RSV infection. We excluded six (1%) duplicate entries. We included 358 (79%) children with RSV-associated mortality originating from 23 different countries across the world (Figure 2, appendix). RSV diagnosis was established most often by immunofluorescence or PCR (appendix). PCR was mostly used in children who died after 2005. Clear seasonality with an annual peak was reported in 18 (78%) of the 23 countries.

117 (33%) children were from low-income or lower middle-income countries, 77 (22%) were from upper middle-income countries, and 164 (46%) were from high-income countries (Table 1). 190 (53%) children were male; we observed no sex differences between countries of different incomes. Children from high-income countries had a higher median age at death than did those in low-income or lower middle-income countries and children from high-income countries had stayed in hospital for a longer period and were more often admitted to a paediatric intensive care unit than those from

Figure 2. Locations of the respiratory syncytial virus-related deaths in young children included in the analysis

Numbers of deaths are given for each country included.

Table 1. Clinical characteristics and prevalence of risk factors in RSV-related child deaths

	Low-income or lower middle-income countries (n = 117)	p value*	Upper middle-income countries (n = 77)	p value†	High-income countries (n = 164)	p value‡
Male sex	58 (50%)	0.976	38 (49%)	0.247	94 (57%)	0.199
Age at death (months)	5.0 [2.3-11.0]	0.973	4.0 [2.0-10.0]	0.023	7.0 [3.6-16.8]	0.006
Younger than 6 months at death	68 (58%)	0.893	44 (57%)	0.014	66 (40%)	0.003
Prematurity§	9 (8%)	0.083	12 (16%)	0.043	45 (27%)	0.000
Gestational age (weeks)	38.0 [38.0-39.0]; n = 22	0.144	38.0 [34.5-38.0]; n = 38	0.250	38.1 [32.1-40.0]; n = 80	0.688
Comorbidity§	33 (28%)	0.008	36 (47%)	0.001	114 (70%)	0.000
Oxygen saturation on room air at hospital admission (%)	92.0 [85.8-97.3]; n = 94	0.022	87.0 [85.0-94.0]; n = 47	0.448	89.0 [76.5-91.0]; n = 37	0.001
Weight for age Z-score of less than -2	64/111 (58%)	0.924	33/58 (57%)	0.370	49/99 (50%)	0.236
Contact with health-care provider before admission to hospital	28/65 (43%)	0.870	17/38 (45%)	0.123	41/68 (60%)	0.047
Time between onset of symptoms and admission (days)	5.0 [3.0-7.0]; n = 71	0.002	3.0 [2.0-4.0]; n = 45	0.387	3.0 [1.0-5.0]; n = 135	0.000
Length of stay in hospital (days)	3.0 [2.0-6.0]	0.000	7.0 [3.0-11.5]	0.000	15.0 [7.0-32.0]; n = 155	0.000
Availability of intensive care unit	28 (24%)	0.000	75 (97%)	0.038	164 (100%)	0.000
Intensive care unit admission	23/116 (20%)	0.000	27/56 (48%)	0.000	152 (93%)	0.000
Mechanical ventilation	23/114 (20%)	0.000	33/60 (55%)	0.000	138/155 (89%)	0.000
Urban living area	22/66 (33%)	0.000	39/40 (98%)	0.867	129/133 (97%)	0.000
At least one sibling present in household	30/39 (77%)	0.122	16/17 (94%)	0.003	62/109 (57%)	0.027
Time of death relative to RSV seasonality						
Death during RSV season	61/79 (77%)	0.004	35/64 (55%)	0.000	122/134 (91%)	0.005
Death within 1 month before or after RSV season	7/79 (9%)	0.050	13/64 (20%)	0.001	7/134 (5%)	0.303

Data are n (%), median [IQR], or n/N (%). Statistical comparisons with Mann-Whitney U test or χ^2 test with p values of less than 0.0167 taken to be significant according to Bonferroni correction for multiple testing. RSV = respiratory syncytial virus. *Low-income or lower middle-income country versus upper middle-income country. †Upper middle-income country versus high-income country. ‡Low-income or lower middle-income country versus high-income country. §Considered absent when missing.

upper middle-income countries or low-income or lower middle-income countries. Median time between onset of symptoms and death was longer in children from high-income countries (21.0 days [IQR 12.0-37.0]) than in those from low-income or lower middle-income countries (9.5 days [7.0-16.8]) or upper middle-income countries (10.0 days [8.0-14.0]; appendix).

We analysed the prevalence of comorbidities in our study (Table 1) and distinguished clinical characteristics of children with and without comorbidities (appendix). Data for comorbidities were missing for some children. Available data showed that comorbidities were present in at least 183 (51%) children. Congenital heart disease was the most frequent comorbid condition identified. The proportion of children with comorbidities was similar during sensitivity analyses when children with missing data (for comorbidities and gestational age) were excluded, except that the proportion with comorbidities in low-income or lower middle-income countries was more than doubled (appendix). Specific information about the presence of comorbidities and prematurity was available in 243 (68%) children. The proportion of missing information about comorbidities and prematurity was similar for children aged younger than 6 months at the time of death (54 [30%] of 178) and older children (61 [34%] of 180).

We analysed how the presence of comorbidities affected age at death (Figure 3). Most healthy term and healthy preterm children were aged younger than 6 months at the time of RSV-related death (appendix). The proportion of children aged younger than 6 months at the time of death was even larger when only children with complete data for comorbidity and gestational age status were included than when all children were included (appendix). Age at death of children with comorbidities from high-income countries was higher than that of those from low-income or middle-income countries.

We analysed the proportion of children with low weight for age. More than half of all children had a weight for age of less than -2 SDs (Table 1). In high-income countries, this finding was mainly explained by a large proportion of preterm children and children with comorbidities. Only eight (28%) of 29 healthy term children in high-income countries had low weight for age compared with eight (89%) of nine healthy preterm children and 33 (54%) of 61 children with comorbidities (appendix). By contrast, in the other two income regions, we found a high proportion (≥48%) of low weight for age in all three children groups, including healthy term infants.

Data for co-infection were scarce. Microbiological data other than RSV diagnosis were sparsely available. We identified co-pathogens in 70 children, with 22 (31%) who tested positive for two or more pathogens besides RSV (data not shown). Positive blood cultures were reported in four (6%) children (two [3%] with Pseudomonas aeruginosa, one [1%] with Klebsiella spp, and one [1%] with Staphylococcus aureus).

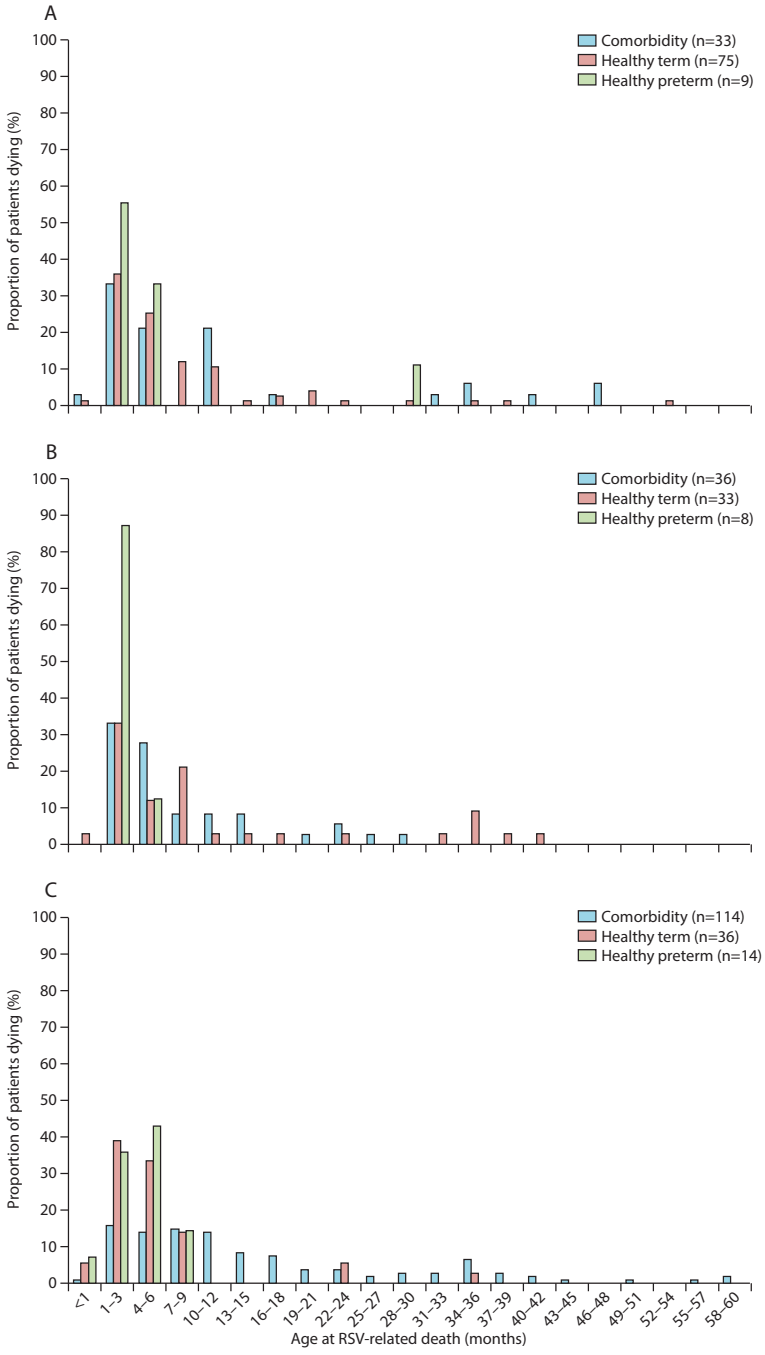

Figure 3. Age distribution at the time of RSV-related death in children
Low-income or lower middle-income countries (A), upper middle-income countries (B), and high-income countries (C). RSV = respiratory syncytial virus.

Data from Argentina (78 [22%]) and Kenya (43 [12%]) contributed to about one third of all RSV-related deaths (121 [34%]). We did a sensitivity analysis excluding these data. The results from these analyses showed a higher age at death for children from high-income countries (10.0 months [4.0-24.0]) than from upper middle-income (4.0 months [2.0-10.0]) or low-income or lower middle-income (4.0 months [2.2-7.0]) countries, which was the same trend as for the main analysis. Other clinical characteristics, including sex and the proportion of prematurity or comorbidities, were also unchanged from the main analysis (data not shown).

Discussion

Severe RSV infection is one of the major causes of global mortality in young children, with 99% of deaths occurring in developing countries in 2015.[5] RSV GOLD is the first descriptive study of global RSV-related mortality using individual case records. We studied clinical characteristics of children who died with RSV in hospitals across the world. We supplemented data from previously published cases of RSV-related in-hospital death with unpublished data by contacting individual investigators, research groups, and networks. Complete clinical information was generally available for children from high-income countries, but clinical data from low-income and middle-income countries were often incomplete. For the first time, we showed that a substantial proportion of in-hospital RSV-related deaths in young children occurred in those with severe comorbidities. In low-income and middle-income countries, most children who died with RSV infection were aged younger than 6 months.

This study describes, to our knowledge, the largest number of children with RSV-related mortality to date. We obtained good global representation, with coverage of all six continents. Clinical profiles of children who die with RSV are essentially different in lower-income countries, where most childhood RSV-associated mortality occurs.[1,5] In low-income or lower middle-income countries, we found that at least 28% of deaths occurred in children with severe comorbidities, such as congenital heart disease. Our data suggest that most RSV-related in-hospital deaths in both low-income and middle-income countries occur in children aged younger than 6 months. Most children with comorbidities (e.g. severe prematurity or congenital heart disease) in these countries might already have died during neonatal age. Additionally, a longer time interval between the onset of symptoms and admission to hospital in low-income countries than in middle-income and high-income countries and a lower proportion of children being admitted to intensive care units and receiving mechanical ventilation in low-income and middle-income countries than in high-income countries suggest that high RSV mortality in these countries might reflect limitations in health-care quality or access to

care rather than specific susceptibility of the children. Young age at death of children with comorbidities in low-income and middle-income countries might have been related to low access to care as well. Paediatric intensive care was available to only 24% of children from low-income or lower middle-income countries and to most children from upper middle-income countries, although only 48% of these children were actually admitted to the intensive care unit.

The role of comorbidities in RSV-related childhood mortality has mostly been described in high-income countries.[10,11,24] The differences in characteristics of children with or without comorbidities were in line with previously published data from high-income settings. Previous studies[10,11,13,14,25,26] have also described a higher age of children with comorbidities at admission or death than of previously healthy children, whereas young age is a risk factor for severe RSV disease in otherwise healthy preterm children.[27] For high-income countries, we substantiate that the age distribution of children who die with RSV infection is substantially different between children with and without severe comorbidities.

Several strategies to prevent RSV infection and RSV-related mortality are being developed.[28] Age at death could influence the effectiveness of perinatal immunisation strategies, including passive immunisation by maternal vaccination during pregnancy and administration of RSV-specific long-half-life monoclonal antibody at birth. Our age distribution data suggest that maternal vaccination will be more effective for healthy term children than for children with comorbidities because antibody concentrations decrease after birth over time. Our data suggest that children with comorbidities will need additional preventive interventions, such as extended passive immunisation or infant vaccination to optimise prevention of RSV-related mortality.

A key strength of this study is that we obtained most previously published cases of RSV-related mortality in children and supplemented this data with data sourced through existing networks, leading to a sample size large enough to draw robust conclusions and do some sensitivity analyses. Second, we verified the quality of each case record through direct interaction with the local researchers. Finally, RSV GOLD is a case series with global representation.

Limitations also deserve discussion. First, although RSV GOLD forms, to our knowledge, the largest case series of RSV-related childhood mortality in hospital, this case series still reflects only a small proportion of all RSV-related deaths worldwide, as most global acute lower respiratory tract infection deaths occur outside of hospital.[29] Data are scarce for RSV-related deaths in the community. Second, in RSV GOLD, only 117 (33%) of the children studied were from low-income or lower middle-income countries, which contrasts with the estimate that 99% of RSV-related mortality occurs in developing countries.[5] Third, RSV GOLD is a case series, which does not allow for calculation of

effect sizes of risk factors for RSV-associated mortality. A population-based study[16] from Buenos Aires reported sepsis and pneumothorax as clinical risk factors for RSV-associated mortality, although sepsis was not pathogen specific. To calculate risk ratios for global RSV-related death with use of original case records, a large prospective population-based study would be needed, which would be challenging given the estimated RSV-attributable mortality rate of 0.86-0.94 per 1000 livebirths.[16] Fourth, our data are limited by several biases. Data are primarily from research sites with availability of diagnostic tests for RSV and RSV diagnostics changing over time, with more sensitive molecular diagnostics used after 2005 than before 2005 (selection bias). Differences in the proportion of available data between income regions could have caused additional selection bias. For example, white blood count and haemoglobin concentration were more often available in low-income regions, which probably reflects measurements in a clinical research setting. Some authors who published data for RSV-related deaths did not respond (response bias), and data for prematurity and comorbidities were often missing, particularly from low-income and middle-income countries (misclassification bias). Misclassification bias could have led to inaccurate interpretation of results. The estimated prevalence of comorbidities in children from low-income or lower middle-income countries is probably an underestimation caused by missing data for comorbidities, but could also be an overestimation caused by selection bias due to a research setting, for example. However, results from our sensitivity analysis supported the main results of our study. Finally, since a substantial proportion of sociodemographic data were missing, these data should be interpreted with caution. For example, the high proportion of RSV-related deaths outside of the RSV season in some countries could reflect inaccurate seasonality data, but could also result from year-round RSV activity, as most of these countries had subtropical climates.

RSV-related infection is an important cause of death in children aged younger than 5 years. Data for RSV-related deaths are scarce and, when available, these data are limited to in-hospital deaths in research settings. Additionally, data for some of the key variables, such as comorbidities and gestational age, are missing. RSV GOLD is being further expanded to collect data for RSV-related deaths from any health facility or community-based study worldwide. Investments to understand the cause of death in young children need to be sustained to inform vaccine policy, particularly in low-income and middle-income settings.

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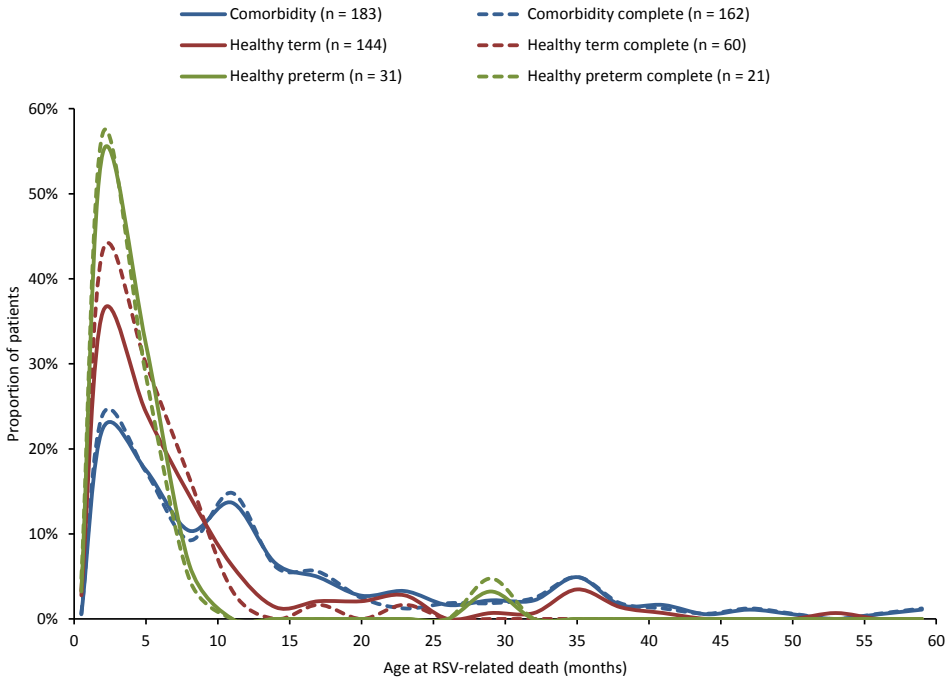
Appendix

Supplementary material



Supplemental Figure 1. Sensitivity analysis for age distribution at RSV-related death excluding children with missing data for comorbidity or prematurity

Supplemental Table 1. Publications that identified research groups that contributed data

Title	Authors	Journal	Year published
Viral etiology of severe pneumonia among Kenyan infants and children	Berkley et al.	JAMA	2010
The impact of prophylaxis on paediatric intensive care unit admissions for RSV infection: a retrospective, single-centre study	Butt et al.	Eur J Pediatr	2011
Intravenous palivizumab and ribavirin combination for respiratory syncytial virus disease in high-risk pediatric patients	Chávez-Bueno et al.	Pediatr Infect Dis J	2007
Epidemiological study of hospitalization associated with respiratory syncytial virus infection in Taiwanese children between 2004 and 2007	Chi et al.	J Formos Med Assoc	2011
Epidemiology of viral-associated acute lower respiratory tract infection among children <5 years of age in a high HIV prevalence setting, South Africa, 2009-2012	Cohen et al.	Pediatr Infect Dis J	2014
Viral epidemiology of respiratory infections among children at a tertiary hospital in Southern Brazil	de-Paris et al.	Rev Soc Bras Med Trop	2014
Incidence and clinical features of hospitalization because of respiratory syncytial virus lower respiratory illness among children less than two years of age in a rural Asian setting	Djelantik et al.	Pediatr Infect Dis J	2003
Case fatality proportions and predictive factors for mortality among children hospitalized with severe pneumonia in a rural developing country setting	Djelantik et al.	J Trop Pediatr	2003
Transaminase levels in ventilated children with respiratory syncytial virus bronchiolitis	Eisenhut et al.	Intensive Care Med	2004
Macronutrients during pregnancy and life-threatening respiratory syncytial virus infections in children.	Ferolla et al.	Am J Respir Crit Care Med	2013
Single, dual and multiple respiratory virus infections and risk of hospitalization and mortality	Goka et al.	Epidemiol Infect	2015
Premorbid factors and outcome associated with respiratory virus infections in a pediatric intensive care unit	Hon et al.	Pediatr Pulmonol	2008
Survey of severe respiratory syncytial virus infection in Kyoto Prefecture from 2003 to 2007	Ito et al.	Pediatr Int	2010
The epidemiology and aetiology of infections in children admitted with clinical severe pneumonia to a university hospital in Rabat, Morocco	Jroundi et al.	J Trop Pediatr	2014
Pre-existing disease is associated with a significantly higher risk of death in severe respiratory syncytial virus infection	K. Thorburn	Arch Dis Child	2009

Title	Authors	Journal	Year published
Burden of respiratory syncytial virus in hospitalized infants and young children in Amman, Jordan	Khuri-Bulos et al.	Scand J Infect Dis	2010
Risk factors associated with death in patients with severe respiratory syncytial virus infection	Lee et al.	J Microbiol Immunol Infect	2014
Epidemiology and risk factors for severe respiratory syncytial virus infections requiring pediatric intensive care admission in Hong Kong children	Leung et al.	Infection	2013
Respiratory syncytial virus: clinical and epidemiological pattern in pediatric patients admitted to a children's hospital between 2000 and 2013	Lucion et al.	Arch Argent Pediatr	2014
Hospital-acquired viral infection increases mortality in children with severe viral respiratory infection	M.C. Spaeder, J.C. Fackler	Pediatr Crit Care Med	2011
Five-year cohort study of hospitalization for respiratory syncytial virus associated lower respiratory tract infection in African children	Madhi et al.	J Clin Virol	2006
Differing manifestations of respiratory syncytial virus-associated severe lower respiratory tract infections in human immunodeficiency virus type 1-infected and uninfected children	Madhi et al.	Pediatr Infect Dis J	2000
Epidemiology of respiratory syncytial virus-associated acute lower respiratory tract infection hospitalizations among HIV-infected and HIV-uninfected South African children, 2010-2011	Moyes et al.	J Infect Dis	2013
Hospitalisation for acute lower respiratory tract infection due to respiratory syncytial virus in Thailand, 2008-2011	Naorat et al.	J Infect Dis	2013
Etiology and epidemiology of viral pneumonia among hospitalized children in rural Mozambique: a malaria endemic area with high prevalence of human immunodeficiency virus	O'Callaghan-Gordo et al.	Pediatr Infect Dis J	2011
Bronchiolitis caused by respiratory syncytial virus in the period from 2003 to 2009	Roglić et al.	Croatian J of infection	2009
Respiratory viruses from hospitalized children with severe pneumonia in the Philippines	Suzuki et al.	BMC Infect Dis	2012

Supplemental Table 2. Origin of data for children younger than five years with RSV-related death

Country	Children with comorbidity (n = 183)	Healthy term children (n = 144)	Healthy preterm children (n = 31)
Low-income			
Mali	3 (2%)	1 (1%)	2 (6%)
Mozambique	0	1 (1%)	0
Lower middle-income			
Indonesia	0	14 (10%)	0
Kenya	17 (9%)	23 (16%)	3 (10%)
Morocco	0	4 (3%)	0
Nicaragua	7 (4%)	8 (6%)	2 (6%)
Pakistan	3 (2%)	1 (1%)	1 (3%)
Philippines	1 (1%)	20 (14%)	0
Zambia	2 (1%)	3 (2%)	1 (3%)
Upper middle-income			
Brazil	8 (4%)	5 (3%)	2 (6%)
Jordan	4 (2%)	5 (3%)	2 (6%)
South Africa	18 (10%)	8 (6%)	4 (13%)
Thailand	6 (3%)	15 (10%)	0
High-income			
Argentina	43 (23%)	26 (18%)	9 (29%)
Australia	5 (3%)	0	0
Canada	4 (2%)	3 (2%)	0
Chile	2 (1%)	0	0
China (Hong Kong & Taiwan)	10 (5%)	0	0
Croatia	1 (1%)	0	0
Germany	1 (1%)	0	0
The Netherlands	7 (4%)	1 (1%)	1 (3%)
United Kingdom	23 (13%)	2 (1%)	1 (3%)
USA	18 (10%)	4 (3%)	3 (10%)

Data are n (%). RSV = respiratory syncytial virus.

Supplemental Table 3. Frequency of RSV diagnostics used over study duration per income region

RSV diagnostic test used	1995 - 2000 (n = 39)	2001 - 2005 (n = 117)	2006 - 2010 (n = 140)	2011 - 2015 (n = 112)
Low-income or lower middle-income country (n = 122)				
PCR	0	2	16	27
Immunofluorescence	0	11	20	27
Enzyme Immuno assay	6	8	0	1
Culture	0	2	0	2
Serology	0	0	0	0
Other	0	0	0	0
Upper middle-income country (n = 122)				
PCR	0	3	30	16
Immunofluorescence	14	6	3	5
Enzyme Immuno assay	0	0	0	0
Culture	0	3	17	1
Serology	0	3	17	1
Other	0	0	0	3
High-income country (n = 164)				
PCR	0	0	6	8
Immunofluorescence	13	62	23	20
Enzyme Immuno assay	2	15	6	0
Culture	4	1	1	0
Serology	0	0	0	0
Other	0	1	1	1

Data are number. RSV = respiratory syncytial virus.

Supplemental Table 4. Additional characteristics and risk factors in RSV-related child deaths

	Low-income or lower middle-income countries (n = 117)	Upper middle-income countries (n = 77)	High-income countries (n = 164)
Comorbidity	33 (28%)	36 (47%)	114 (70%)
Genetic or chromosomal disease	4 (3%)	3 (4%)	31 (19%)
Down's syndrome	3 (3%)	2 (3%)	10 (6%)
Congenital heart disease	15 (13%)	9 (12%)	43 (26%)
Neurological disease	4 (3%)	5 (6%)	40 (24%)
Chronic lung disease	1 (1%)	0	45 (27%)
Airway abnormality	0	1 (1%)	7 (4%)
Primary immunodeficiency	0	1 (1%)	7 (4%)
Malignancy	0	0	2 (1%)
HIV infection	5 (4%)	18 (23%)	0
Tuberculosis infection	1 (1%)	2 (3%)	0
Other underlying disease	9 (8%)	4 (5%)	4 (2%)
Biliary atresia	0	1 (1%)	0
Liver disease	0	1 (1%)	0
Renal disease	0	1 (1%)	2 (1%)
Immunosuppression	5 (4%)	0	0
Congenital abnormality	3 (3%)	0	0
Adenoid cyst	1 (1%)	0	0
Metabolic disorder	0	1 (1%)	2 (1%)
Clinical symptoms			
Cough	105 (90%)	67 (87%)	43 (26%)
Difficult breathing	105 (90%)	48 (62%)	68 (41%)
Fast breathing*	72 (62%)	41 (53%)	46 (28%)
Chest indrawing	83 (71%)	28 (36%)	36 (22%)
Central cyanosis	15 (13%)	8 (10%)	13 (8%)
Severe respiratory distress	27 (23%)	4 (5%)	46 (28%)
Inability to drink	25 (21%)	16 (21%)	31 (19%)
Lethargy or unconsciousness	38 (32%)	11 (14%)	14 (9%)
RSV diagnostic test used			
PCR	45 (38%)	49 (64%)	14 (9%)
Immunofluorescence	58 (50%)	28 (36%)	118 (72%)
Enzyme immunoassay	15 (13%)	0	23 (14%)
Culture	4 (3%)	21 (27%)	6 (4%)
Serology	0	21 (27%)	0
Other	0	3 (4%)	3 (2%)
RSV diagnostic specimen used			
Nasal swab	0	0	3 (2%)
Nasopharyngeal swab	60 (51%)	36 (47%)	13 (8%)
Nasopharyngeal aspirate	22 (19%)	35 (45%)	119 (73%)
Wash	35 (30%)	3 (4%)	0
Blood	0	21 (27%)	0
Other	2 (2%)	0	7 (4%)

	Low-income or lower middle-income countries (n = 117)	Upper middle-income countries (n = 77)	High-income countries (n = 164)
White blood cell count			
< 6000 cu/mm	14 (12%)	6 (8%)	11 (7%)
6000-25000 cu/mm	81 (69%)	32 (42%)	49 (30%)
> 25000 cu/mm	13 (11%)	1 (1%)	9 (5%)
Unknown	6 (5%)	38 (49%)	95 (58%)
Haemoglobin level			
< 5 g/dL	3 (3%)	0	1 (1%)
5-8 g/dL	16 (14%)	5 (6%)	3 (2%)
8-10 g/dL	47 (40%)	7 (9%)	30 (18%)
> 10 g/dL	41 (35%)	32 (42%)	28 (17%)
Unknown	7 (6%)	33 (43%)	102 (62%)
Length of stay at intensive care unit (days)	3.5 [1.8-8.5]; n = 38	11.0 [6.0-22.0]; n = 15	13.0 [6.0-31.5]; n = 132
Duration of mechanical ventilation (days)	4.0 [(1.0-9.3]; n = 22	8.0 [2.0-17.0]; n = 19	12.0 [5.0-27.3]; n = 126
Time between onset of symptoms and death (days)	9.5 [7.0-16.8]; n = 72	10.0 [8.0-14.0]; n = 45	21.0 [12.0-37.0]; n = 135
Type of feeding during first four months of life			
Exclusive breast feeding	23 (20%)	5 (6%)	11 (7%)
Mixed breast bottle feeding	16 (14%)	6 (8%)	18 (11%)
Exclusive bottle feeding	2 (2%)	6 (8%)	25 (15%)
Unknown	76 (65%)	60 (78%)	110 (67%)
Parental smoking	5/39 (13%)	5/15 (33%)	16/45 (36%)
Maternal education (highest level attended)			
No education	8 (7%)	4 (5%)	0
Primary school	8 (7%)	8 (10%)	4 (2%)
Secondary school	17 (15%)	9 (12%)	32 (20%)
University level	3 (3%)	1 (1%)	3 (2%)
Unknown	81 (69%)	55 (71%)	125 (76%)
Daycare attendance	3/38 (8%)	0/17	5/117 (4%)

Data are n (%), median [IQR], or n/N (%). RSV = respiratory syncytial virus. *For age younger than 2 months, at least 60 breaths per min; for age 2-11 months, at least 50 breaths per min; for age 1-5 years, at least 40 breaths per min.

Supplemental Table 5A. Clinical characteristics and risk factors in RSV-related child deaths from low-income or lower middle-income countries

	Children with comorbidity (n = 33)	**Healthy term children (n = 75)**	**Healthy preterm children (n = 9)**
Male sex	15 (45%)	38 (51%)	5 (56%)
Age at death (months)	5.0 [2.5-11.5]	5.0 [2.4-10.0]	3.0 [1.0-5.5]
Younger than 6 months at death	17 (52%)	44 (59%)	7 (78%)
Prematurity	0	NA	9 (100%)
Gestational age (weeks)	38.5 [38.0-39.8]; n = 8	38.0 [38.0-38.0]; n = 11	31.0 [31.0-31.5]; n = 3
Comorbidity			
Genetic or chromosomal disease	4 (12%)		
Congenital heart disease	15 (45%)		
Neurological disease	4 (12%)		
Chronic lung disease	1 (3%)		
Airway abnormality	0		
Primary immunodeficiency	0		
Malignancy	0		
HIV infection	5 (15%)		
Tuberculosis infection	1 (3%)		
Other underlying disease	9 (27%)		
Clinical symptoms			
Cough	30 (91%)	68 (91%)	7 (78%)
Difficult breathing	29 (88%)	68 (91%)	8 (89%)
Fast breathing	27 (82%)	39 (52%)	6 (67%)
Chest indrawing	27 (82%)	49 (65%)	7 (78%)
Central cyanosis	4 (12%)	9 (12%)	2 (22%)
Severe respiratory distress	9 (27%)	14 (19%)	4 (44%)
Inability to drink	9 (27%)	12 (16%)	4 (44%)
Lethargy or unconsciousness	9 (27%)	28 (37%)	1 (11%)
Oxygen saturation on room air at hospital admission (%)	92.0 [82.0-98.0]; n = 31	93.0 [87.0-97.0]; n = 54	92.0 [86.0-97.0]; n = 9
Weight for age Z-score of less than -2	23/31 (74%)	35/73 (48%)	6/7 (86%)
RSV diagnostic test used			
PCR	9 (27%)	32 (43%)	4 (44%)
Immunofluorescence	24 (73%)	29 (39%)	5 (56%)
Enzyme immunoassay	1 (3%)	14 (19%)	0
Culture	2 (6%)	2 (3%)	0
Serology	0	0	0
Other	0	0	0
RSV diagnostic specimen used			
Nasal swab	0	0	0
Nasopharyngeal swab	19 (58%)	37 (49%)	4 (44%)
Nasopharyngeal aspirate	7 (21%)	14 (19%)	1 (11%)
Wash	7 (21%)	26 (35%)	2 (22%)
Blood	0	0	0
Other	1 (3%)	0	1 (11%)
Contact with health-care provider before admission to hospital	12/25 (48%)	14/35 (40%)	2/5 (40%)

	Children with comorbidity (n = 33)	**Healthy term children (n = 75)**	**Healthy preterm children (n = 9)**
Time between onset of symptoms and admission (days)	3.0 [2.0-7.8]; n = 26	5.5 [3.0-7.0]; n = 38	4.0 [3.0-5.0]; n = 7
Length of stay in hospital (days)	4.0 [1.5-8.0]	3.0 [2.0-6.0]	3.0 [1.5-8.0]
Availability of intensive care unit	11 (33%)	14 (19%)	3 (33%)
Intensive care unit admission	9 (27%)	11/74 (15%)	3 (33%)
Mechanical ventilation	9 (27%)	11/72 (15%)	3 (33%)
Urban living area	9/21 (43%)	10/41 (24%)	3/4 (75%)
At least one sibling present	11/13 (85%)	15/20 (75%)	4/6 (67%)
Time of death relative to RSV seasonality			
Death during RSV season	21/25 (84%)	34/48 (71%)	6/6 (100%)
Death within 1 month before or after RSV season	1/25 (4%)	6/48 (13%)	0

Data are n (%), median [IQR], or n/N (%). RSV = respiratory syncytial virus. NA = not applicable.

Supplemental Table 5B. Clinical characteristics and risk factors in RSV-related child deaths from upper middle-income countries

	Children with comorbidity (n = 36)	**Healthy term children (n = 33)**	**Healthy preterm children (n = 8)**
Male sex	17 (47%)	17 (52%)	4 (50%)
Age at death (months)	4.2 [2.3-10.8]	7.0 [2.3-16.5]	2.0 [2.0-2.8]
Younger than 6 months at death	21 (58%)	15 (45%)	8 (100%)
Prematurity	4/27 (15%)	NA	8 (100%)
Gestational age (weeks)	38.0 [37.0-38.0]; n = 21	39.0 [37.5-40.0]; n = 10	31.0 [29.0-35.0]; n = 7
Comorbidity			
Genetic or chromosomal disease	3 (8%)		
Congenital heart disease	9 (25%)		
Neurological disease	5 (14%)		
Chronic lung disease	0		
Airway abnormality	1 (3%)		
Primary immunodeficiency	1 (3%)		
Malignancy	0		
HIV infection	18 (50%)		
Tuberculosis infection	2 (6%)		
Other underlying disease	4 (11%)		
Clinical symptoms			
Cough	30 (83%)	31 (94%)	6 (75%)
Difficult breathing	17 (47%)	25 (76%)	6 (75%)
Fast breathing	22 (61%)	16 (48%)	3 (38%)
Chest indrawing	16 (44%)	7 (21%)	5 (63%)
Central cyanosis	3 (8%)	4 (12%)	1 (13%)
Severe respiratory distress	2 (6%)	1 (3%)	1 (13%)
Inability to drink	10 (28%)	5 (15%)	1 (13%)
Lethargy or unconsciousness	6 (17%)	4 (12%)	1 (13%)
Oxygen saturation on room air at hospital admission (%)	87.0 [84.5-90.3]; n = 25	92.0 [86.5-96.5]; n = 17	87.0 [78.5-91.5]; n = 5
Weight for age Z-score of less than -2	18/29 (62%)	12/25 (48%)	3/4 (75%)
RSV diagnostic test used			
PCR	18 (50%)	27 (82%)	4 (50%)
Immunofluorescence	18 (50%)	6 (18%)	4 (50%)
Enzyme immunoassay	0	0	0
Culture	6 (17%)	15 (45%)	0
Serology	6 (17%)	15 (45%)	0
Other	1 (3%)	2 (6%)	0
RSV diagnostic specimen used			
Nasal swab	0	0	0
Nasopharyngeal swab	13 (36%)	21 (64%)	2 (25%)
Nasopharyngeal aspirate	20 (56%)	10 (30%)	5 (63%)
Wash	1 (3%)	2 (6%)	0
Blood	6 (17%)	15 (45%)	0
Other	0	0	0
Contact with health-care provider before admission to hospital	9/18 (50%)	6/16 (38%)	2/4 (50%)

	Children with comorbidity (n = 36)	**Healthy term children (n = 33)**	**Healthy preterm children (n = 8)**
Time between onset of symptoms and admission (days)	3.0 [2.0-4.8]; n = 24	4.0 [1.0-6.0]; n = 15	2.5 [1.8-4.0]; n = 6
Length of stay in hospital (days)	7.0 [3.3-11.8]	6.0 [3.0-10.0]	9.0 [5.3-15.3]
Availability of intensive care unit	36 (100%)	31 (94%)	8 (100%)
Intensive care unit admission	11/28 (39%)	10/20 (50%)	6/8 (75%)
Mechanical ventilation	16/22 (73%)	15/32 (47%)	2/6 (33%)
Urban living area	23/23 (100%)	10/11 (91%)	6/6 (100%)
At least one sibling present	8/8 (100%)	6/6 (100%)	2/3(67%)
Time of death relative to RSV seasonality			
Death during RSV season	16/31 (52%)	16/27 (59%)	3/6 (50%)
Death within 1 month before or after RSV season	9/31 (29%)	2/27 (7%)	2/6 (33%)

Data are n (%), median [IQR], or n/N (%). RSV = respiratory syncytial virus. NA = not applicable.

Supplemental Table 5C. Clinical characteristics and risk factors in RSV-related child deaths from high-income countries

	Children with comorbidity (n = 114)	**Healthy term children (n = 36)**	**Healthy preterm children (n = 14)**
Male sex	64 (56%)	21 (58%)	9 (64%)
Age at death (months)	11.0 [5.0-23.3]	4.0 [2.0-6.0]	3.8 [2.0-5.3]
Younger than 6 months at death	29 (25%)	26 (72%)	11 (79%)
Prematurity	31/105 (30%)	NA	14 (100%)
Gestational age (weeks)	38.1 [32.2-40.0]; n = 62	40.0 [39.0-40.0]; n = 11	30.0 [28.0-34.0]; n = 7
Comorbidity			
Genetic or chromosomal disease	31 (27%)		
Congenital heart disease	43 (38%)		
Neurological disease	40 (35%)		
Chronic lung disease	45 (39%)		
Airway abnormality	7 (6%)		
Primary immunodeficiency	7 (6%)		
Malignancy	2 (2%)		
HIV infection	0		
Tuberculosis infection	0		
Other underlying disease	4 (4%)		
Clinical symptoms			
Cough	34 (30%)	6 (17%)	3 (21%)
Difficult breathing	54 (47%)	9 (25%)	5 (36%)
Fast breathing	39 (34%)	6 (17%)	1 (7%)
Chest indrawing	28 (25%)	6 (17%)	2 (14%)
Central cyanosis	11 (10%)	1 (3%)	1 (7%)
Severe respiratory distress	37 (32%)	6 (17%)	3 (21%)
Inability to drink	2 (2%)	5 (14%)	1 (7%)
Lethargy or unconsciousness	12 (11%)	2 (6%)	0
Oxygen saturation on room air at hospital admission (%)	88.0 [75.8-92.8]; n = 28	89.0 [75.0-90.0]; n = 7	NA
Weight for age Z-score of less than -2	33/61 (54%)	8/29 (28%)	8/9 (89%)
RSV diagnostic test used			
PCR	13 (11%)	1 (3%)	0
Immunofluorescence	75 (66%)	31 (86%)	12 (86%)
Enzyme immunoassay	19 (17%)	3 (8%)	1 (7%)
Culture	3 (3%)	0	3 (21%)
Serology	0	0	0
Other	2 (2%)	1 (3%)	0
RSV diagnostic specimen used			
Nasal swab	1 (1%)	2 (6%)	0
Nasopharyngeal swab	11 (10%)	2 (6%)	0
Nasopharyngeal aspirate	81 (71%)	28 (78%)	10 (71%)
Wash	0	0	0
Blood	0	0	0
Other	6 (5%)	1 (3%)	0
Contact with health-care provider before admission to hospital	33/55 (60%)	7/10 (70%)	1/3 (33%)

	Children with comorbidity (n = 114)	**Healthy term children (n = 36)**	**Healthy preterm children (n = 14)**
Time between onset of symptoms and admission (days)	2.0 [1.0-3.0]; n = 92	5.0 [3.0-10.0]; n = 31	2.0 [2.0-3.0]; n = 12
Length of stay in hospital (days)	15.0 [6.0-34.6]	19.0 [9.0-32.0]	11.0 [6.5-23.5]
Availability of intensive care unit	114 (100%)	36 (100%)	14 (100%)
Intensive care unit admission	105 (92%)	34 (94%)	13 (93%)
Mechanical ventilation	94/107 (88%)	32/35 (91%)	12/13 (92%)
Urban living area	88/91 (97%)	31/32 (97%)	10/10 (100%)
At least one sibling present	40/70 (57%)	17/30 (57%)	5/9 (56%)
Time of death relative to RSV seasonality			
Death during RSV season	81/91 (89%)	31/32 (97%)	10/11 (91%)
Death within 1 month before or after RSV season	5/91 (5%)	1/32 (3%)	1/11 (9%)

Data are n (%), median [IQR], or n/N (%). RSV = respiratory syncytial virus. NA = not applicable.

Supplemental Table 6. Clinical characteristics and risk factors in RSV-related child deaths excluding children with missing data for comorbidity or prematurity

	Low-income or lower middle-income countries (n = 45)	Upper middle-income countries (n = 48)	High-income countries (n = 150)
Male sex	21 (47%)	23 (48%)	85 (57%)
Age at death (months)	4.0 [2.0-11.0]	3.0 [2.0-7.4]	7.0 [3.3-16.0]
Children with comorbidity	4.5 [2.0-11.3]; n = 30	4.0 [2.0-8.8]; n = 27	11.0 [5.0-22.0]; n = 105
Healthy term children	4.5 [1.5-8.3]; n = 12	2.8 [1.5-8.0]; n = 14	4.0 [1.9-5.3]; n = 34
Healthy preterm children	3 [2-15.5]; n = 3	2 [2-2]; n = 7	4.0 [2.0-5.0]; n = 11
Younger than 6 months at death	26 (58%)	35 (73%)	63 (42%)
Prematurity	3 (7%)	11 (23%)	42 (28%)
Gestational age (weeks)	38.0 [38.0-39.0]; n = 21	38.0 [35.0-38.0]; n = 35	39.0 [34.1-40.0]; n = 77
Comorbidity	30 (67%)	27 (56%)	105 (70%)
Genetic or chromosomal disease	3 (7%)	3 (6%)	28 (19%)
Congenital heart disease	12 (27%)	4 (8%)	41 (27%)
Neurological disease	3 (7%)	4 (8%)	38 (25%)
Chronic lung disease	1 (2%)	0	41 (27%)
Airway abnormality	0	1 (2%)	7 (5%)
Primary immunodeficiency	0	0	7 (5%)
Malignancy	0	0	2 (1%)
HIV infection	5 (11%)	16 (33%)	0
Tuberculosis infection	1 (2%)	2 (4%)	0
Other underlying disease	9 (20%)	3 (6%)	3 (2%)
Clinical symptoms			
Cough	41 (91%)	41 (85%)	40 (27%)
Difficult breathing	40 (89%)	25 (52%)	65 (43%)
Fast breathing	33 (73%)	22 (46%)	46 (31%)
Chest indrawing	34 (76%)	25 (52%)	36 (24%)
Central cyanosis	8 (18%)	7 (15%)	13 (9%)
Severe respiratory distress	16 (36%)	4 (8%)	44 (29%)
Inability to drink	13 (29%)	15 (31%)	31 (21%)
Lethargy or unconsciousness	11 (24%)	8 (17%)	13 (9%)
Oxygen saturation on room air at hospital admission (%)	92.0 [82.0-98.0]; n = 41	87.0 [85.5-89.3]; n = 29	89.0 [76.5-91.0]; n = 37
Weight for age Z-score of less than -2	31/43 (72%)	20/36 (56%)	48/97 (49%)
RSV diagnostic test used			
PCR	14 (31%)	27 (56%)	14 (9%)
Immunofluorescence	31 (69%)	21 (44%)	111 (74%)
Enzyme immunoassay	1 (2%)	0	23 (15%)
Culture	1 (2%)	0	1 (1%)
Serology	0	0	0
Other	0	2 (4%)	3 (2%)

	Low-income or lower middle-income countries (n = 45)	Upper middle-income countries (n = 48)	High-income countries (n = 150)
RSV diagnostic specimen used			
Nasal swab	0	0	3 (2%)
Nasopharyngeal swab	20 (44%)	14 (29%)	13 (9%)
Nasopharyngeal aspirate	12 (27%)	30 (63%)	117 (78%)
Wash	7 (16%)	2 (4%)	0
Blood	0	21 (44%)	0
Other	1 (2%)	0	7 (5%)
Contact with health-care provider before admission to hospital	15/36 (42%)	8/18 (44%)	41/68 (60%)
Time between onset of symptoms and admission (days)	4.0 [3.0-7.0]; n = 39	3.0 [2.0-4.0]; n = 43	3.0 [1.0-5.0]; n = 131
Length of stay in hospital (days)	4.0 [1.5-10.0]	7.0 [4.0-11.0]	17.0 [7.8-32.3]
Availability of intensive care unit	24 (53%)	48 (100%)	150 (100%)
Intensive care unit admission	17 (38%)	19/46 (41%)	138 (92%)
Mechanical ventilation	17/43 (40%)	12/31 (39%)	129/143 (90%)
Urban living area	19/32 (59%)	32/33 (97%)	127/131 (97%)
At least one sibling present in household	19/26 (73%)	13/14 (93%)	62/109 (57%)
Time of death relative to RSV seasonality			
Death during RSV season	28/37 (76%)	25/36 (69%)	119/131 (91%)
Death within 1 month before or after RSV season	2/37 (5%)	7/36 (19%)	7/131 (5%)

Data are n (%), median [IQR], or n/N (%). RSV = respiratory syncytial virus.

Supplemental Table 7. Frequency of age at RSV-related death per income region by comorbidity status

Age at death	Children with comorbidity (n = 183)	Healthy term children (n = 144)	Healthy preterm children (n = 31)
Low-income or lower middle-income country (n = 117)			
0-1 months of age	1 (3%)	1 (1%)	0
1-2 months of age	2 (6%)	9 (12%)	3 (33%)
2-3 months of age	5 (15%)	9 (12%)	1 (11%)
3-4 months of age	4 (12%)	9 (12%)	1 (11%)
4-5 months of age	4 (12%)	6 (8%)	0
5-6 months of age	1 (3%)	10 (13%)	2 (22%)
6-12 months of age	8 (24%)	18 (24%)	1 (11%)
12-24 months of age	2 (6%)	8 (11%)	0
24-60 months of age	6 (18%)	5 (7%)	1 (11%)
Upper middle-income country (n = 77)			
0-1 months of age	0	1 (3%)	0
1-2 months of age	1 (3%)	3 (9%)	0
2-3 months of age	8 (22%)	4 (12%)	6 (75%)
3-4 months of age	3 (8%)	4 (12%)	1 (13%)
4-5 months of age	7 (19%)	2 (6%)	0
5-6 months of age	2 (6%)	1 (3%)	1 (13%)
6-12 months of age	7 (19%)	9 (27%)	0
12-24 months of age	6 (17%)	3 (9%)	0
24-60 months of age	2 (6%)	6 (18%)	0
High-income country (n = 164)			
0-1 months of age	1 (1%)	2 (6%)	1 (7%)
1-2 months of age	2 (2%)	5 (14%)	0
2-3 months of age	11 (10%)	8 (22%)	4 (29%)
3-4 months of age	4 (4%)	1 (3%)	1 (7%)
4-5 months of age	5 (4%)	9 (25%)	4 (29%)
5-6 months of age	6 (5%)	1 (3%)	1 (7%)
6-12 months of age	31 (27%)	7 (19%)	3 (21%)
12-24 months of age	26 (23%)	0	0
24-60 months of age	28 (25%)	3 (8%)	0

Data are n (%). RSV = respiratory syncytial virus.

Supplemental RSV GOLD questionnaire

RSV diagnosis

1.1 Diagnostic test used (several answers possible)

- o PCR
- o immunofluorescence
- o enzyme immuno assay
- o culture
- o serology
- o other: …

1.2 Specimen (several answers possible)

- o nasal swab
- o nasopharyngeal swab
- o nasopharyngeal aspirate
- o wash
- o blood
- o other: …

Age

2.1 Age at moment of death … months (0-59)

2.2 Date of death: month/year (Jan-Dec)/(1995-2015)

Basic patient characteristics

3.1 Gender

- o male
- o female
- o unknown

3.2 Severe underlying disease
(if yes, several answers possible) yes/no/unknown

- o congenital heart disease
- o chronic lung disease
- o immunodeficiency
- o genetic/chromosomal disease
- o neuromuscular disease
- o neurodevelopmental disease
- o airway abnormality
- o malignancy
- o other: …

3.3 Prematurity yes/no/unknown
< 37 completed weeks of gestation

3.4 Length/height at admission (use cm or in) … centimetre (cm)
Or current length if out of hospital patient … inches (in)

3.5 Weight at admission (use kg or lbs): … kilogram (kg)
Or current weight if out of hospital patient … pounds (lbs)

3.6 Current type of feeding
Current feeding if child is < 4 months of age. If child is > 4 months of age report feeding in first 4 months of life

- o exclusive breast feeding
- o mixed breast & bottle feeding
- o exclusive bottle feeding
- o unknown
- o other: …

Hospital admission

4.1 Hospitalisation yes/no/unknown

4.2 Length of stay in hospital (if applicable) … days

4.3 Intensive care admission yes/no/unknown, if yes … days

4.4 Mechanical ventilation — yes/no/unknown, if yes … days

4.5 Time interval between onset of RSV related symptoms and hospital admission — … days

4.6 Contact with other health-care providers before hospitalisation — yes/no/unknown

4.7 Place of death; in hospital? — yes/no/unknown

4.8 Time interval between onset of RSV related symptoms and death — … days

Clinical characteristics

5.1 Clinical symptoms present (several answers possible):

- o cough
- o difficult breathing
- o fast breathing (age < 2 months, ≥ 60 breaths; age 2-11 months, ≥ 50/min; age 1-5 years, ≥ 40/min)
- o chest indrawing
- o central cyanosis
- o severe respiratory distress (e.g. grunting, very severe chest indrawing)
- o inability to breastfeed or drink, vomiting everything
- o lethargy, reduced level of consciousness or convulsions

5.2 Co-infection present — yes/no/unknown

if y:

- o malaria
- o HIV/AIDS
- o TB
- o other: …

5.3 Oxygen saturation (SpO2) on room air upon hospital admission …%

5.4 WBC count

- o <6000/cu mm
- o 6000-25.000/cu mm
- o >25.000/cu mm
- o unknown

5.5 Haemoglobin level

- o <5 g/dL
- o 5-<8 g/dL
- o 8-10 g/dL
- o >10 g/dL
- o unknown

5.6 Other respiratory virus or bacteria present in respiratory sample

yes/no/unknown

if yes (several answers possible):

- o Influenza A/B
- o Para-influenza virus
- o Human metapneumovirus
- o Adenovirus
- o Rhinovirus
- o Streptococcus pneumoniae
- o Haemophilus influenzae
- o Mycoplasma pneumoniae
- o other pathogen or sample: …

5.7 Hospital-acquired RSV infection yes/no/unknown

History

6.1 Gestational age … weeks estimated by:
LMP/ultrasonography/unknown/both

6.2 Birth weight (use kg or lbs) … kilogram
… pounds

6.3 Immunisation status (specify per vaccine)

Fully means according to local immunization schedule and age. Partially means 1 or more dose(s) missed.

PCV fully/partially/not/unknown
Hib fully/partially/not/unknown
MMR fully/partially/not/unknown
BCG fully/partially/not/unknown
DTP fully/partially/not/unknown

Socio-demographic characteristics

7.1 RSV seasonality
Estimated start and end of RSV season during calendar year

- o year round (no clear peak season)
- o yearly on average 1 peak season
  start: … end: …
- o yearly on average 2 peak seasons
  start: … end: … of peak season 1
  start: … end: … of peak season 2
- o timing of peak season varies heavily per year
- o unknown
- o other: …

7.2 Other children in household — yes/no/unknown

7.3 Day care attendance — yes/no/unknown

7.4 Living area — rural/urban/unknown

7.5 Maternal education (highest level attended)

- o uneducated
- o primary school level
- o secondary school level
- o university level
- o unknown

7.6 Parental smoking — yes/no/unknown

7.7 Comments or additional information

3

Potential impact of maternal vaccination on life-threatening respiratory syncytial virus infection during infancy

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Abstract

Background

Respiratory syncytial virus (RSV) infection is an important cause of infant mortality. Here, we estimated the potential impact of maternal vaccination against RSV on life-threatening RSV infection in infants.

Methods

We developed a mathematical model for maternal vaccine-induced antibody dynamics and used characteristics of a maternal RSV vaccine currently in phase 3 of clinical development. The model was applied to data from two cohorts of children younger than 12 months with RSV-related paediatric intensive care unit (PICU) admission in the United Kingdom (n = 370) and the Netherlands (n = 167), and a cohort of 211 children younger than 12 months with RSV-related in-hospital death from 20 countries worldwide.

Results

Our model predicted that, depending on vaccine efficiency, maternal vaccination at 30 weeks' gestational age could have prevented 62-75% of RSV-related PICU admissions in the United Kingdom and 76-87% in the Netherlands. For the global mortality cohort, the model predicted that maternal vaccination could have prevented 29-48% of RSV-related in-hospital deaths. Preterm children and children with comorbidities were predicted to benefit less than (healthy) term children.

Conclusions

Maternal vaccination against RSV may substantially decrease life-threatening RSV infections in infants.

Introduction

Respiratory syncytial virus infection (RSV) is an important cause of morbidity and mortality in young children.[1,2] Globally, it is estimated that 48 000-74 500 children aged younger than five years died in-hospital with RSV-related lower respiratory tract infection in 2015.[2] About 99% of RSV-related childhood mortality occurs in developing countries.[2] Most RSV-related mortality occurs during the first year of life.[3–6] In our recent global case series study of 358 children with RSV-related in-hospital death, median age at death varied from 4 to 7 months depending on income region (upper middle-income vs. high-income countries, respectively).[6] Preterm children and children with comorbidities such as congenital heart disease or chronic lung disease are at increased risk for severe RSV infection or even fatal RSV infection.[7–10]

Maternal vaccination is currently being considered for RSV prevention in young children.[11] Maternal vaccination will only provide temporary protection due to an age-dependent decrease of maternally-acquired protective antibodies after birth.[12,13] For example, serological studies from Bangladesh and Kenya reported maternally-acquired protective antibodies against RSV to be present only up to four months after birth.[14–16] Similarly, maternal vaccination against influenza and pertussis provides protection during the first two to three months of life.[12,17] As transplacental antibody transfer becomes efficient only from the third trimester of pregnancy onward, maternal vaccination may provide limited protection for preterm infants.[18–20] To date, the potential impact of a maternal RSV vaccine on RSV-related mortality in young children is unknown. In this observational, retrospective study, we developed a mathematical model for maternal vaccine-induced antibody dynamics, taking into account transplacental antibody transfer rates and antibody decline after birth.[15,18,21–24] We applied this model to data from two retrospective cohorts of children with RSV-related paediatric intensive care unit (PICU) admission and a previously published cohort of children with RSV-related in-hospital death[6] and predicted the percentage of life-threatening RSV infections potentially prevented by maternal vaccination.

Methods

To predict the percentage of life-threatening RSV infections potentially prevented by maternal vaccination we developed a mathematical model for maternal vaccine-induced antibody dynamics, taking into account transplacental transfer rates of protective antibodies during pregnancy and antibody decline in new-born children.

Maternal vaccine-induced antibodies

Vaccination of pregnant women against RSV infection induces an increase in maternal anti-RSV antibodies. For simplicity, maternal vaccine-induced RSV-specific antibody levels were modelled as an exponential increase between day 7 and day 21 post-vaccination, and were assumed to stay constant afterward.[15,21,22] We hence modelled maternal vaccine-induced RSV-specific antibody levels (a_m in µg/ml) as follows (Figure 1A):

$$if\ \ t < 7,\ \ a_m(t) = a_{m0}$$
$$if\ 7 \le t \le 21,\ \ a_m(t) = a_{m0}\ e^{(t-7)\log(f)/14}$$
$$if\ \ t > 21,\ \ a_m(t) = a_{m0}\ f,$$

where t is the time in days since vaccination, a_{m0} the natural level of maternal RSV-specific antibodies in µg/ml, and f the vaccine-induced fold increase in maternal RSV-specific antibodies (later referred to as vaccine efficiency).

Transfer of maternal antibodies

During pregnancy, maternal immunoglobulin G (IgG) antibodies are transferred to the foetus by transplacental transport. Transplacental transfer is thought to increase during pregnancy to become most efficient during the third trimester, and at term foetal IgG concentrations typically even exceed maternal IgG levels.[18–20,25] Based on published data on maternal and foetal IgG antibody levels at different time points during pregnancy (section 5 and figure 3 (IgG1) of Palmeira et al.[26] which are based on figure 2 of Malek et al.[18]), we chose to model maternal IgG antibody transfer with an exponential function, as it gave the best description of the experimental data.[18] The parameters of this function were estimated using the function *lm* of R (version 3.3.2) after a log-transformation of the data and the best fit of this model to the experimental data was found for:

$$r(t) = e^{-4.97+0.13t},$$

where r is the foetus-to-mother IgG transfer ratio and t is time in days since the beginning of pregnancy (Figure 1B).

Foetal antibody levels after birth

We assumed that maternally-derived RSV-specific antibody levels in umbilical cord blood at birth (a_{cb} in µg/ml) can be calculated directly from the RSV-specific antibody levels in the mother and the foetus-to-mother IgG transfer ratio at time t_b, the gestational age (i.e. time of birth) in days, using the following function:

$$a_{cb}(t_b) = a_m(t_b)\,.\,r(t_b).$$

After birth, maternally-acquired RSV-specific antibody levels in the new-born were assumed to decrease with a half-life $t_{1/2}$. The RSV-specific antibody levels of new-born children (a_c in µg/ml) can therefore be described as follows (Figure 1C):

$$a_c(t) = a_{cb}(t_b)\left(\frac{1}{2}\right)^{t/t_{1/2}},$$

where t is the age of the new-born child in days after birth.

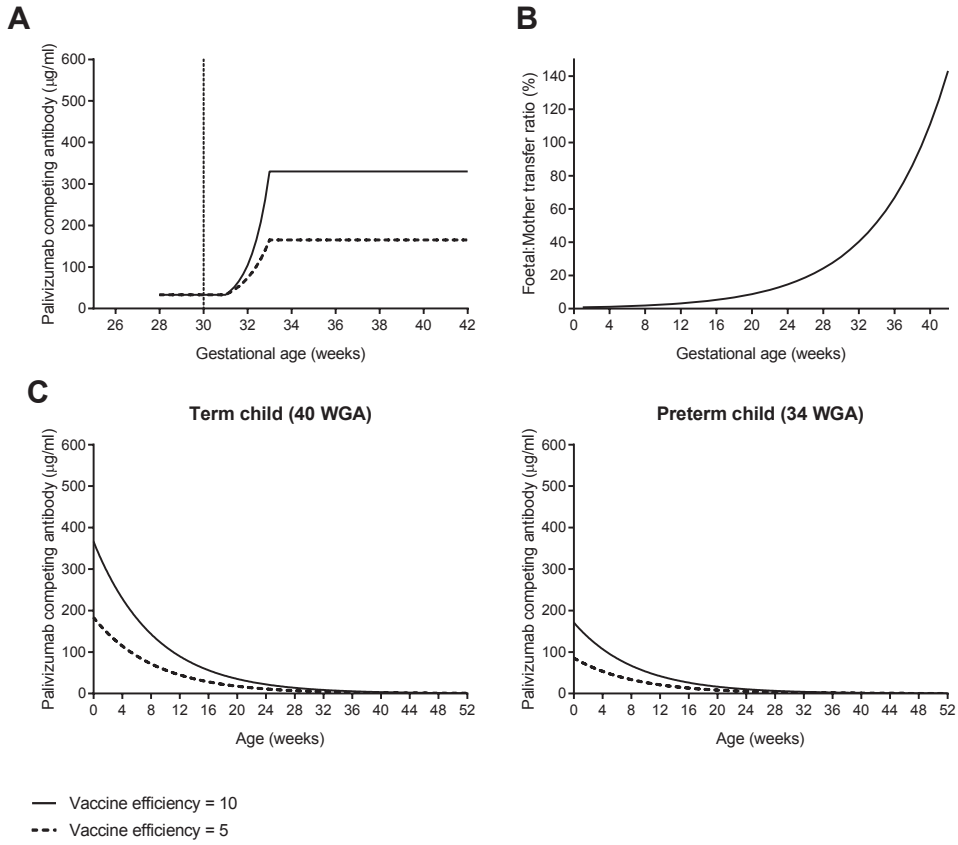

Figure 1. Antibody levels after maternal vaccination in mother and child
(A) Vaccine-induced maternal antibody levels for maternal vaccination at 30 weeks' gestational age (WGA). (B) Transplacental antibody transfer during pregnancy. (C) Maternally-acquired antibody levels after birth for maternal vaccination at 30 WGA for a term child (born at 40 WGA) and a preterm child (born at 34 WGA).

Model parameterization

Similar antibody dynamics for maternal vaccine-induced RSV-specific IgG and palivizumab competing antibody (PCA) have been reported[21,27] and as more data are available for PCA we decided to parameterize our model on PCA. A natural PCA level a_{m0} of 33 μg/ml was used based on the phase-2 trial studying the safety and immunogenicity of a recombinant RSV fusion protein nanoparticle vaccine (RSV F vaccine) candidate in non-pregnant women of childbearing age.[21] PCA levels after vaccination against RSV have been reported to be 6.9-7.9-fold higher than the natural PCA level, depending on vaccine dosing.[21] Based on these values, we considered two vaccine efficiencies (*f*) of 5 and 10 in our simulations. RSV-specific antibody half-life after birth was reported to be 41 days by maternal RSV F vaccine manufacturers[23], which is in close agreement with reported values of 36-38 days in clinical studies measuring cord blood and infant maternally-acquired RSV-specific antibody levels.[15,28] A child PCA level of 40 μg/ml was considered as the protective threshold against life-threatening RSV infection.[24,29,30]

Study population

We applied our model to three independent, retrospective cohorts of patients. Anonymised secondary patient data were obtained through retrospective review of medical records. The first and second cohort consisted of children aged younger than 12 months with community-acquired RSV infection admitted to the PICU for mechanical ventilation, who all survived. The first cohort consisted of 370 children admitted to a PICU in the United Kingdom (UK) between 2002-2014 and the second cohort consisted of 167 children admitted to two PICUs in the Netherlands between 2008-2015. None of the children had received palivizumab during infancy. In the third cohort, children were selected from a retrospective case series study describing global, in-hospital, RSV-related mortality in 358 children aged younger than five years.[6] All children aged younger than 12 months with available data for prematurity were included. This resulted in a study population of 211 children with RSV-related in-hospital death from 20 countries. In the second and third cohort, when the exact gestational age was missing (i.e. for 60 term children in the Dutch PICU cohort and for 19 preterm and 85 term children in the mortality cohort) it was imputed to 34 or 40 weeks of gestation for preterm or term children respectively, based on median values of children with complete gestational age data.

For each cohort, we defined the following subgroups: children with comorbidities, healthy term children (born without comorbidities, at 37 weeks' gestational age or later) and healthy preterm children (born without comorbidities, earlier than 37 weeks' gestational age). For the global mortality cohort, countries were categorized as i) high income, ii) upper middle income, and iii) lower middle or low income, on the basis of the World Bank classifications for 2016.[31]

Prediction of life-threatening RSV infections prevented by maternal vaccination

To predict the percentage of children with life-threatening RSV infection that could be prevented by maternal vaccination, we assumed that all mothers were vaccinated during pregnancy and used gestational age and age at RSV-related outcome (PICU admission or death). The general recommendation for maternal vaccination against pertussis is to vaccinate during the late-second or early-third trimester of pregnancy[32,33], while future maternal RSV vaccination is suggested during the third trimester[23]. Therefore, to evaluate the effect of timing of maternal vaccination, we first considered a large window for maternal vaccination between 22 and 40 weeks of gestation and then reported results on maternal vaccination at 30 weeks of gestation as a reflection of the recommendation to vaccinate during the early-third trimester of pregnancy.

For each child, we predicted the antibody level at birth taking into account timing of maternal vaccination, vaccine efficiency and gestational age at birth and then modelled antibody level decline until the observed time of RSV-related outcome (age at PICU admission or death). We considered that a RSV-related outcome would be prevented (or at least postponed) if the simulated vaccine-derived protective antibody level was above the protective threshold at the time of outcome.

Results

Study population

Among the 370 children with RSV-related PICU admission for mechanical ventilation in the United Kingdom, 107 (29%) were born preterm and median age at the time of PICU admission was 49 days (InterQuartileRange (IQR) 30-88) (Table 1). In the Dutch cohort of children admitted to the PICU, 35 (21%) were born preterm and median age at the time of PICU admission was 38 days (IQR 27-63). The prevalence of comorbidity was reported to be 28% (n = 102) for the PICU cohort from the United Kingdom and 11% (n = 19) for the PICU cohort from the Netherlands.

Among the 211 children with global, RSV-related in-hospital death, 51 (24%) were born preterm and median age at the time of RSV-related death was 4 months (IQR 2-7). In the global mortality cohort, comorbidities were reported for 103 (49%) children, and 103 (49%) children were from high-income countries (Table 1). Median length of stay in hospital for children with RSV-related in-hospital death was 9 days (IQR 4-22, n = 205). For each cohort, the distributions for gestational age (GA) and age at RSV-related outcome (PICU admission or death) are shown in Figure 2.

Table 1. Clinical characteristics in children with RSV-related PICU admission or in-hospital death

	PICU cohort from the United Kingdom (n = 370)	PICU cohort from the Netherlands (n = 167)	Global mortality cohort (n = 211)
Male sex	222 (60%)	94 (56%)	116 (55%)
Age at RSV-related outcome* (days)	49 [30-88]	38 [27-63]	122 [61-213]
Prematurity (<37 WGA)	107 (29%)	35 (21%)	51 (24%)
Gestational age (weeks)	40 [36-40]	40 [37-40]	40 [37-40]
Comorbidity status			
Comorbidity	102 (28%)	19 (11%)	103 (49%)
Healthy and term	182 (49%)	117 (70%)	78 (37%)
Healthy and preterm	86 (23%)	31 (19%)	30 (14%)
Country of origin			
Low-income or lower middle-income			60 (28%)
Upper middle-income			48 (23%)
High-income	370 (100%)	167 (100%)	103 (49%)

Data are n (%) or median [IQR]. RSV = respiratory syncytial virus. PICU = paediatric intensive care unit. *PICU admission or death.

Percentage of life-threatening RSV infections prevented by maternal vaccination

We applied the mathematical model for maternal vaccine-induced antibody levels (see Methods) to individual patient data (gestational age and age at RSV-related outcome) from the three study cohorts. We hence predicted the percentage of outcomes that could have been prevented (or at least postponed) if mothers had been vaccinated between 22 and 40 weeks of gestation for two vaccine efficiencies (f = 5 and 10, see Methods).

The model predicted that the percentage of cases prevented would be highest if the maternal vaccine would be administered between 22 and approximately 30 weeks' GA and would start to decrease for maternal vaccination after 30 weeks' GA as a result of the time required to reach maternal antibody peak levels (Figure 2). The highest predicted percentage prevented was lower than 100% (80% in the UK cohort and 88% in the Dutch cohort with a vaccine efficiency of 10) and observed for maternal vaccination during the second trimester (between 22 and 26 weeks' GA). This resulted from the prevalence of prematurity and from age at PICU admission at which antibodies had already declined below the protective threshold. In the global mortality cohort, maternal vaccination during the second trimester would have prevented even fewer cases (maximum of 50% for a vaccine efficiency of 10). This lower percentage resulted from the higher age of children at RSV-related death (compared to the average age at PICU admission in the other two cohorts), leading to a larger proportion of RSV-related deaths occurring after antibody levels had already declined below the protective

threshold. Therefore, vaccine efficiency had a larger impact on the percentage of cases prevented for the global mortality cohort than for the PICU cohorts.

According to our model, maternal vaccination at 30 weeks' GA would prevent at least 62% of RSV-related PICU admissions (62-75% in the United Kingdom and 76-87% in the Netherlands) and 29-48% of RSV-related in-hospital deaths, depending on vaccine efficiency (Figure 2).

Impact of maternal vaccination on high-risk populations

We performed subgroup analyses to study the potential impact of maternal vaccination on high-risk populations such as preterm children, children with comorbidities and children from lower income regions. In preterm children, median age at the time of PICU

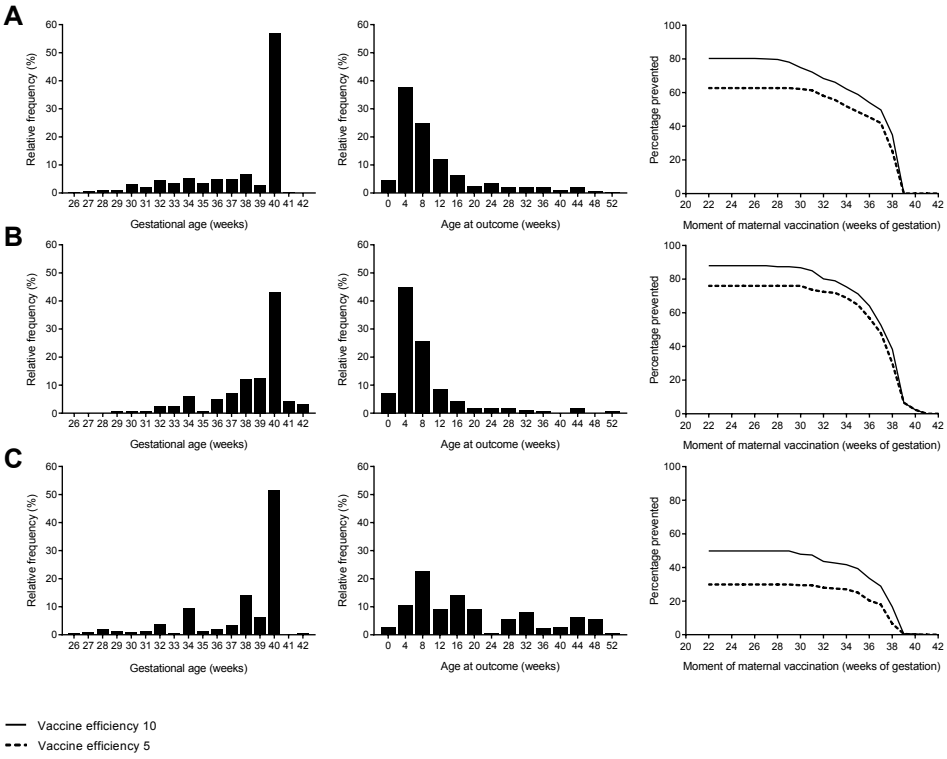

Figure 2. Distribution of gestational age and age at outcome and the predicted percentage of children with life-threatening RSV infection prevented by maternal vaccination for each cohort

Gestational age in weeks (left), age at outcome in weeks (middle) and predicted percentage of prevented cases are plotted for (A) PICU cohort from the United Kingdom (n = 370), (B) PICU cohort from the Netherlands (n = 167) and (C) Global mortality cohort (n = 211). The predicted percentages of prevented cases were calculated for two vaccine efficiencies (f = 10 solid line and f = 5 dashed line).

admission was 53 days (IQR 32-78 and 37-80 for the United Kingdom and Netherlands respectively) and median age at the time of RSV-related in-hospital death was 122 days (IQR 61-183) (Supplemental Table 1). The model predicted that maternal vaccination, even if administered during the second trimester (i.e. 22-26 weeks' GA), would prevent a smaller percentage of preterm than term children in each cohort (Figure 3). This is a direct consequence of the reduced transplacental antibody transfer relative to the time of birth for preterm children compared to term children. For preterm children, maternal vaccination at 30 weeks' GA could have prevented 36-51% of RSV-related PICU admissions in the United Kingdom (vs. 73-85% in term children), 46-71% in the

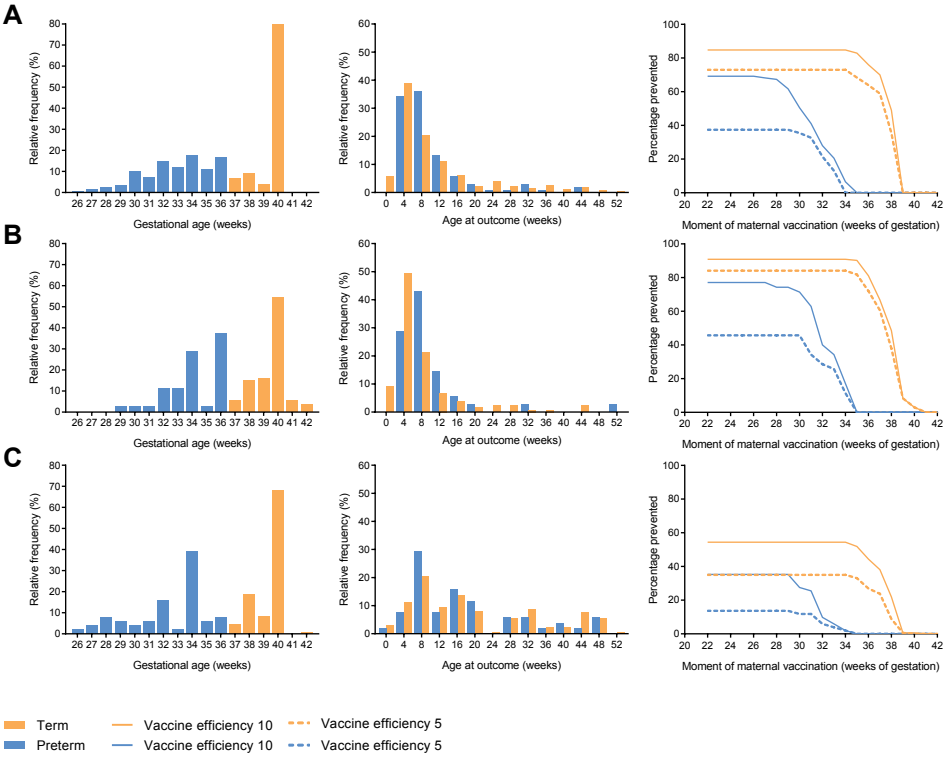

Figure 3. Distribution of gestational age and age at outcome and the predicted percentage of children with life-threatening RSV infection prevented by maternal vaccination for each cohort stratified by prematurity status

Gestational age in weeks (left), age at outcome in weeks (middle) and predicted percentage of prevented cases are plotted for (A) PICU cohort from the United Kingdom (Term n = 263, Preterm n = 107), (B) PICU cohort from the Netherlands (Term n = 132, Preterm n = 35) and (C) Global mortality cohort (Term n = 160, Preterm n = 51). Term children are in orange and Preterm in blue. The predicted percentages of prevented cases were calculated for two vaccine efficiencies (f = 10 solid line and f = 5 dashed line).

Netherlands (vs. 84-91% in term children), and 12-28% of RSV-related in-hospital deaths (vs. 35-54% in term children). Later vaccination would further decrease the percentage of prevented cases among preterm children.

Children with comorbidities tended to be older at the time of RSV-related PICU admission than healthy term children, whereas age at the time of RSV-related in-hospital death was similar for children with comorbidities and healthy term children (Supplemental Table 1). We found that children with comorbidities would benefit less from maternal vaccination than healthy term children regardless of the timing of maternal vaccination (Figure 4). For the PICU cohort from the United Kingdom, maternal vaccination at 30 weeks'

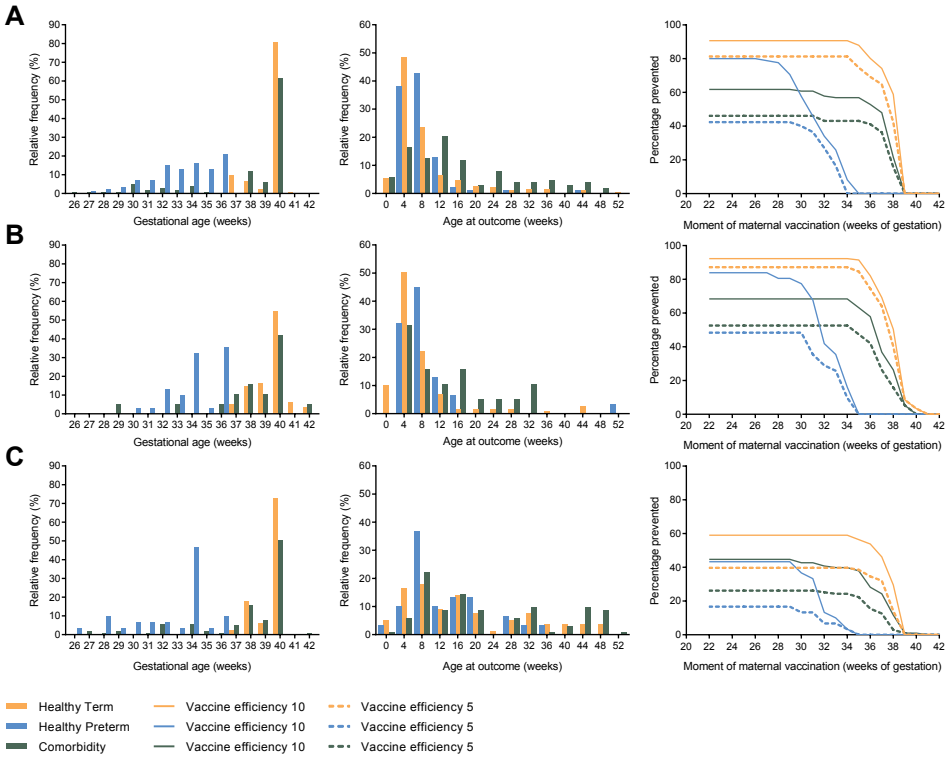

Figure 4. Distribution of gestational age and age at outcome and the predicted percentage of children with life-threatening RSV infection prevented by maternal vaccination for each cohort stratified by comorbidity status

Gestational age in weeks (left), age at outcome in weeks (middle) and predicted percentage of prevented cases (right) are plotted for (A) PICU cohort from the United Kingdom (Healthy term n = 182; Healthy preterm n = 86; Comorbidity n = 102), (B) PICU cohort from the Netherlands (Healthy term n = 117; Healthy preterm n = 31; Comorbidity n = 19) and (C) Global mortality cohort (Healthy term n = 78; Healthy preterm n = 30; Comorbidity n = 103). Healthy term children are in orange, Healthy preterm in blue and Children with comorbidities in green. The predicted percentages of prevented cases were calculated for two vaccine efficiencies (f = 10 solid line and f = 5 dashed line).

GA would have prevented PICU admission in 46-61% of children with comorbidities (53-68% in the Dutch cohort) compared to 81-91% of healthy term children (87-92% in the Dutch cohort). For the global mortality cohort, maternal vaccination at 30 weeks' GA would have prevented RSV-related in-hospital death in 26-43% of children with comorbidities compared to 40-59% of healthy term children.
In children with RSV-related death from lower income regions (28% of the global mortality cohort), the predicted percentage of prevented in-hospital deaths was 30-50% for maternal vaccination at 30 weeks' GA and similar to that in children from other income regions (data not shown).

Discussion

We have developed a mathematical model to predict the percentage of children with life-threatening RSV infection during the first year of life that may be prevented by maternal vaccination. The model was calibrated using vaccine characteristics of a maternal RSV vaccine currently in phase 3 of clinical development and was applied to individual patient data for RSV-related PICU admission and death. The model predicts that maternal vaccination against RSV could substantially decrease life-threatening RSV infections in infants. Preterm children and children with comorbidities, those at increased risk for severe RSV, were predicted to benefit less from a maternal RSV vaccine than term and healthy children.
We assumed in the model that transplacental maternal antibody transfer increases during pregnancy and becomes most efficient during the third trimester and at term, as described by Malek et al.[18] This assumption is in agreement with previous studies on transplacental transfer in preterm infants for other pathogens[19], and with a study examining the effectiveness of maternal pertussis vaccination in England, which observed limited benefit of maternal vaccination for preterm compared to term children.[34] In contrast, a recent study describing maternal RSV antibody levels in comparison to cord blood levels in 26 preterm infants found similar antibody levels in preterm and term children[25], suggesting that transplacental antibody transfer may already be efficient before the third trimester. Given the increased risk for severe RSV infection in preterm children and the relatively high prevalence of prematurity in lower income regions[35], where most RSV-related childhood mortality occurs[2], having a better knowledge of transplacental antibody transfer is essential to improve the prediction of the impact of maternal vaccination.
A strength of our study was the use of characteristics of a maternal RSV vaccine currently in development and of patient data from three independent cohorts. We were able to predict the percentage of prevented (or at least postponed) cases for the overall

study population and for subgroups more at risk for life-threatening RSV infection, such as preterm children or children with comorbidities. This model was developed to study the impact of a specific maternal RSV vaccine, but may be used for other maternal vaccines by calibrating for specific vaccine characteristics.

Although we have based our model on current biological knowledge, some of the assumptions need further discussion. We did not incorporate RSV protective antibodies acquired by natural RSV infection during pregnancy or vaccine-induced protective antibodies in breastmilk. These factors may influence the antibody level in children and, therefore, protection against RSV. In addition, we did not incorporate local vaccine coverage, RSV transmission patterns, risk of RSV-related disease or disease severity. A more classical approach such as the compartment model recently published by Hogan et al.[36] in combination with our approach would provide more insights into vaccine effectiveness at a population level. We applied the model to three cohorts of children with life-threatening RSV infection which has introduced selection bias. For example, high-risk groups, such as preterm children, likely have been overrepresented given their increased risk for severe RSV infection, whereas the exclusion of children who had received palivizumab prophylaxis may have resulted in underrepresentation of children with severe prematurity. Imputation of missing gestational age data and the inclusion of preterm children (20%) in the subgroup of children with comorbidities may also have influenced our results. The percentage of cases prevented, as predicted by our model, should therefore not be directly extrapolated to the general population. In addition, results may not be generalizable to lower income regions as the majority of children in our study were from high-income countries.

Several model assumptions may have resulted in an overestimation of the impact of maternal vaccination on life-threatening RSV infection. First, we assumed that antibody levels in the mother would exponentially increase from 7 days post-vaccination and reach a maximal level at 21 days post-vaccination. This level was assumed to stay constant through the rest of pregnancy.[15] If, however, antibody levels in the mother actually declined over time, antibody transfer to the foetus would be reduced and our model would overestimate the percentage of cases prevented by maternal vaccination. Additionally, if maternal antibody levels were to decline over time, this could affect the optimal timing of maternal vaccination. Second, our model did not consider reduced transplacental transfer caused by maternal comorbidities, such as malaria or hypergammaglobulinemia.[12] Third, in our model for maternal vaccine-induced antibody dynamics, we assumed that the antibody half-life was 41 days, based on available maternal RSV vaccine phase-2 trial data[21], which is higher than reported by others.[37] When we considered an antibody half-life as short as 20 days, the percentage of prevented cases would be reduced (e.g. 11-24% instead of 35-56% of RSV-related

in-hospital deaths for maternal vaccination at 30 weeks' GA). Fourth, we assumed full protection from life-threatening RSV infection as long as a child's predicted antibody levels remained above the protective threshold of 40 µg/ml.[24,29,30] However, the correlation between antibody levels and protection from RSV disease has not been well defined.[15,38,39] Fifth, our results describe the situation in which all mothers would have been vaccinated during pregnancy. When we considered a vaccine coverage of 60%, based on the observed vaccine coverage for maternal pertussis vaccination in the United Kingdom and Belgium[40,41], the percentage of prevented cases would be substantially lower (e.g. 42-48% and 50-54% instead of 62-75% and 76-87% of RSV-related PICU admissions in the United Kingdom and the Netherlands respectively for maternal vaccination at 30 weeks' GA). Finally, protection from RSV infection was assumed to result in prevention of RSV-related PICU admission or death, whereas for some children it may merely have postponed these RSV-related outcomes.

In summary, our mathematical model suggests that maternal vaccination against RSV could substantially decrease the number of life-threatening RSV infections in infants. In order to inform policy makers about the need for additional preventive interventions after birth for high-risk groups, such as preterm children, future studies on maternal vaccination should provide accurate data for transplacental antibody transfer per week of gestation, antibody half-life in new-born children and the protective antibody threshold.

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Appendix

Supplementary material

Supplemental Table 1. Age at RSV-related PICU admission or in-hospital death stratified by prematurity and comorbidity status

	PICU cohort from the United Kingdom		PICU cohort from the Netherlands		Global mortality cohort	
	Age (days)	**N**	**Age (days)**	**N**	**Age (days)**	**N**
Prematurity status*						
Term	45 [28-92]	263	36 [23-62]	132	122 [61-213]	160
Preterm	53 [32-78]	107	53 [37-80]	35	122 [61-183]	51
Comorbidity status						
Comorbidity	89 [48-171]	102	80 [35-151]	19	122 [61-213]	103
Healthy and term	40 [24-63]	182	35 [24-58]	117	122 [46-183]	78
Healthy and preterm	49 [31-63]	86	50 [37-67]	31	69 [61-152]	30

Data are median [IQR]. RSV = respiratory syncytial virus. PICU = paediatric intensive care unit.
*Prematurity defined as <37 weeks' gestational age.

4

Respiratory syncytial virus prevention and asthma in healthy preterm infants: a randomised controlled trial

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Abstract

Background

Respiratory syncytial virus (RSV) infection is associated with subsequent wheeze and asthma. We previously reported on the causal relationship between prevention of RSV infection during infancy and reduced frequency of subsequent wheeze using a double-blind, randomised, placebo-controlled trial (MAKI). We continued follow-up and analysed the effect of RSV prevention during infancy on asthma and lung function at age 6 years.

Methods

We studied 429 infants born at 32-35 weeks of gestation between 2008-10 who had randomly received either palivizumab for RSV immunoprophylaxis or placebo during the RSV season of their first year of life. After the first year of follow-up, single, assessor-blind follow-up of children continued until they were aged 6 years. Primary outcomes were parent-reported current asthma and forced expiratory volume in 0.5 s ($FEV_{0.5}$). The trial is registered in the ISRCTN registry, number ISRCTN73641710.

Findings

395 (92%) of 429 participants completed this 6-year follow-up study. Parent-reported current asthma was reported in 28 (14.1%) of 199 children in the RSV prevention group and 47 (24.0%) of 196 children in the placebo group (absolute risk reduction [ARR] 9.9%, 95% CI 2.2 to 17.6). The difference in current asthma, which was a composite endpoint, was due to a difference in infrequent wheeze (one to three episodes in the past year; 12 [6.0%] of 199 vs. 26 [13.4%] of 194, ARR 7.4%, 95% CI 1.5 to 13.2). $FEV_{0.5}$ percentage predicted values were similar between the RSV prevention group (89.1% [SD 10.6]) and placebo group (90.1% [11.1]), with a mean difference of 1.0 (95% CI -1.3 to 3.3). The proportion of children with current physician-diagnosed asthma was similar between the RSV prevention group (19 [10.3%] of 185) and placebo group (18 [9.9%] of 182), with an ARR of -0.4 (95% CI -6.5 to 5.8).

Interpretation

In otherwise healthy preterm infants, this single-blind, randomised, placebo-controlled trial showed that RSV prevention did not have a major effect on current asthma or lung function at age 6 years. Future research will inform on the effect of RSV prevention on asthma at school age in the general population.

Research in context

Evidence before this study

Respiratory syncytial virus (RSV) infection during infancy has been associated with asthma in later childhood. However, the nature of the relationship between RSV infection and asthma is unclear. We searched PubMed for clinical trials that assessed the link between RSV prevention and subsequent development of asthma. We searched for articles published in English up to Aug 11, 2017, using search terms related to RSV infection and prevention, wheeze, and asthma. We found one randomised trial from the USA showing no effect of RSV prevention by motavizumab on medically attended wheezing up to age 3 years in native American children. One observational case-control study from Japan showed that RSV prevention by palivizumab in otherwise healthy preterm children reduced subsequent physician-diagnosed recurrent wheezing up to age 6 years.

Added value of this study

We have previously reported on the causal relationship between RSV infection and recurrent wheeze in otherwise healthy preterm infants using a double-blind, randomised, placebo-controlled trial. We continued single, assessor-blind follow-up of trial participants and analysed the effect of RSV prevention during infancy on asthma and lung function at age 6 years. We found that RSV prevention in otherwise healthy preterm infants reduced the risk of parent-reported asthma at age 6 years, which was mainly explained by differences in proportions of participants with infrequent wheeze. RSV prevention did not affect the risk of physician-diagnosed asthma or results of lung function tests at age 6 years.

Implications of all the available evidence

To the best of our knowledge, this is the first study designed to establish the causal relationship between RSV infection during infancy and asthma up to school age in otherwise healthy preterm infants. RSV prevention did not have a major effect on current asthma or lung function at age 6 years, which might have implications for future RSV preventive interventions in terms of cost-effectiveness.

Introduction

Respiratory syncytial virus (RSV) infection is a notable cause of morbidity and mortality in young children,[1,2] and RSV-related lower respiratory tract infection has been associated with subsequent wheeze and asthma at school age.[3–10] With more than 358 million people affected by asthma in 2015, it is one of the most common chronic diseases globally and creates substantial disability in children.[11]

The nature of the relationship between RSV infection during infancy and asthma in later childhood is unclear. Some observational cohort studies have shown an increased risk of asthma after RSV infection during infancy,[3–8,10] but others have shown no clear relationship.[12–14] However, these studies were not designed to provide evidence for causality. A recent observational case-control study that followed preterm children from Japan up to the age of 6 years showed that RSV prophylaxis reduced the incidence of recurrent wheezing, but not of atopic asthma.[7] A randomised trial done in healthy term infants in the USA studied the effect of RSV prevention by motavizumab on severe RSV infection and medically attended wheezing up to age 3 years, but did not examine long-term asthma development.[15] To date, no randomised clinical trial using RSV prophylaxis has assessed the causal link between RSV infection during infancy and subsequent development of asthma or abnormal lung function.

We previously reported 1-year follow-up results of a multicentre, double-blind, randomised, placebo-controlled trial (MAKI) assessing the causal link between RSV infection during infancy and recurrent wheeze.[16] We showed that, compared with placebo, RSV prevention by the monoclonal antibody palivizumab reduced the total number of wheezing days in the first year of life by 61% in otherwise healthy preterm infants. Single, assessor-blind follow-up of trial participants continued. In this study we assessed the effect of RSV prevention during infancy on asthma and lung function at age 6 years in the same patient population, in line with WHO and SAGE recommendations.[17,18]

Methods

Study design and participants

In the MAKI trial, we enrolled otherwise healthy preterm infants, born at 32 weeks and 1 day to 35 weeks and 6 days of gestation.[16] Infants were younger than 6 months at the start of the RSV season and were randomly assigned in a 1:1 ratio to receive palivizumab for RSV immunoprophylaxis or placebo. Randomisation was stratified according to gestational age and masking was secured by an independent pharmacist who had generated a permuted-block randomisation list. Parents recorded airway

symptoms and airway-related physician attendance or medication use in a daily log until the first birthday of their infant. Details about the study design and study protocol have been previously described ISRCTN73641710).[16]

In this study, children were followed up until age 6 years. To ensure continued study participation, parents received regular newsletters. Parents were invited to complete a questionnaire on respiratory symptoms when their child was aged 3 years and to participate in this follow-up study when their child was aged 6 years. Study assessments were done at the paediatric lung function laboratory of the University Medical Center Utrecht or at the child's home by the same research team and equipment, if preferred by parents. The study team was re-masked after the first year of follow-up. Thus, study assessments were conducted by researchers who were unaware of the child's assigned treatment group. The randomisation code was kept by an independent physician until 6-year follow-up was completed. Parents who had been unmasked in 2011 were instructed not to reveal treatment allocation to the researchers at follow-up.

Parents provided separate written informed consent for their child to participate in the follow-up study. The institutional review board at the University Medical Center Utrecht approved the study, which was part of an amended protocol. The study was conducted according to the principles of the Declaration of Helsinki (2000) and to Good Clinical Practice guidelines, including yearly monitoring.

Procedures

Following a recent recommendation from a US Food and Drug Administration (FDA)-National Institutes of Health (NIH) workshop on RSV vaccine development,[19] we measured patient-reported outcomes with a parent-completed asthma questionnaire and lung function to show effectiveness of RSV prevention in preventing asthma. Using a patient-reported outcome for asthma was also suggested by an academic expert group, concluding that parent-reported questionnaires are commonly used in seminal studies and are feasible for longitudinal studies.[20]

At age 3 years, a trial-specific questionnaire on respiratory health was completed by parents, including questions about wheeze since the child's first birthday and current use of airway medication (bronchodilators or inhaled corticosteroids). Data collected at age 3 years were analysed together with data collected at age 6 years after the research team had been unmasked.

At age 6 years, the International Study of Asthma and Allergies in Childhood questionnaire[21] was done, supplemented with trial-specific questionnaires on respiratory health. We contacted general practitioners of trial participants to obtain patient information on medically attended asthma symptoms. From this information, we assessed the presence of current physician-diagnosed asthma. All respiratory function

assessments were done according to the American Thoracic Society-European Respiratory Society guidelines[22] and spirometry results were converted to percentage predicted values using Dutch normative values adjusted for sex, age, and height.[23] In addition to spirometry, the fraction of exhaled nitric oxide was measured in exhaled breath and the resistance of the respiratory system was assessed by the interrupter technique (Rint).[22] Median resistance of the respiratory system was calculated from at least eight acceptable interruptions (appendix).
Serum total IgE concentration and allergen-specific IgE for house dust mite, birch, mugwort, timothy, *Aspergillus fumigatus*, dog, and cat were measured using ImmunoCAP tests (Thermo Fisher Scientific, Uppsala, Sweden).

Outcomes
The first primary outcome was current asthma, defined as parent-reported wheeze in the past 12 months or use of asthma medication in the past 12 months, or both (appendix). Parent-reported wheeze in the past 12 months was categorised into infrequent (one to three episodes) or frequent (more than three episodes). The second primary outcome was forced expiratory volume in 0.5 s ($FEV_{0.5}$), assessed by spirometry. $FEV_{0.5}$ was chosen instead of FEV_1 as the primary outcome because of the young age of study participants.[22] All outcomes were at age 6 years unless otherwise specified.
Secondary outcomes were FEV_1, forced vital capacity (FVC), forced expiratory flow between 25% and 75% of vital capacity (FEF_{25-75}), $FEV_{0.5}$:FVC ratio, FEV_1:FVC ratio, $FEV_{0.5}$ and FEV_1 after administration of a bronchodilator, fraction of exhaled nitric oxide, and resistance of the respiratory system.
Secondary questionnaire-based outcomes were wheeze at ages 1-3 years, use of asthma medication at age 3 years, nocturnal cough in the past 12 months, use of oral corticosteroids for respiratory symptoms ever, medically attended respiratory infection in the past 12 months (otitis, upper respiratory tract infection, pharyngitis, pseudocroup, or lower respiratory tract infection), eczema, allergic rhinitis, and food allergy. Eczema was defined as a positive response to the question “Has your child ever experienced eczema?” Allergic rhinitis was defined as a positive response to the question “Has your child ever experienced hay fever?”
Secondary laboratory-based outcomes were total IgE and allergic sensitisation defined as an allergen-specific IgE concentration of at least 0.35 kU per L for at least one of the tested allergens.
Current physician-diagnosed asthma was a post-hoc endpoint, defined as physician-diagnosed asthma or bronchial hyper-reactivity in the past 12 months. We used this composite endpoint because it is common clinical practice in the Netherlands to postpone asthma diagnosis until age 7 years or older.

Statistical analysis

All analyses were done on an intention-to-treat basis. The primary and secondary outcomes were prespecified, and a detailed description of the analyses is in the appendix. Current physician-diagnosed asthma was defined post-hoc. Post-hoc subgroup analyses were done to assess the influence of atopic status, intertester variability, differences over study duration (2014-16), and study site (home visit or research centre). Study groups were compared by calculating absolute risk reductions (ARR), relative risk reductions, or mean differences with 95% CI. If applicable, between-group differences were assessed using the χ^2 test, Fisher's exact, t test, or Mann-Whitney U test. MAKI was powered to establish a clinically relevant difference in current asthma of 9% or more and a clinically relevant difference in $FEV_{0.5}$ percentage predicted of 7% or more. Complete case analysis was done if less than 10% of data were missing. Otherwise, we did multiple imputation and compared results of complete case analysis with results from the multiple imputation. Approximately 100 baseline characteristics and relevant outcome variables were used for imputation. Statistical analyses were done with SPSS statistical software, version 22.0, except for multiple imputation, which was done with R, version 3.1.1 using the mice package (appendix).

Results

429 infants were recruited to the original study between April and December of each year between 2008 and 2010, and assigned to receive either palivizumab (n = 214) or placebo (n = 215). Baseline characteristics at randomisation have been published previously and were well balanced between study groups.[16] 199 participants in the palivizumab group and 196 participants in the placebo group were included in the follow-up at age 6 years (Figure 1). 342 (87%) of these 395 children successfully completed spirometry. 103 (26%) of 395 participants had their study measurements taken at home. Mean age at 6 years follow-up was similar between the groups (5.8 years [SD 0.3] vs. 5.9 years [0.3]; appendix).

The proportion of children with current parent-reported asthma was lower in the RSV prevention group (28 [14.1%] of 199) than in the placebo group (47 [24.0%] of 196 participants; ARR 9.9% [95% CI 2.2 to 17.6]; Table 1). Current asthma was a composite outcome of wheeze and use of asthma medication in the past 12 months. Fewer children reported wheeze in the RSV prevention group (23 [11.6%] of 199) than in the placebo group (39 [19.9%] of 196; ARR 8.3% [95% CI 1.2 to 15.5]), while similar proportions of participants used asthma medication (18 [9.0%] of 199 in the RSV prevention group vs. 25 [12.8%] of 195 in the placebo group; ARR 3.8% [95% CI -2.4 to 9.9]; Table 2). Of the participants who reported wheeze in the past 12 months, 12 (52.2%) of the

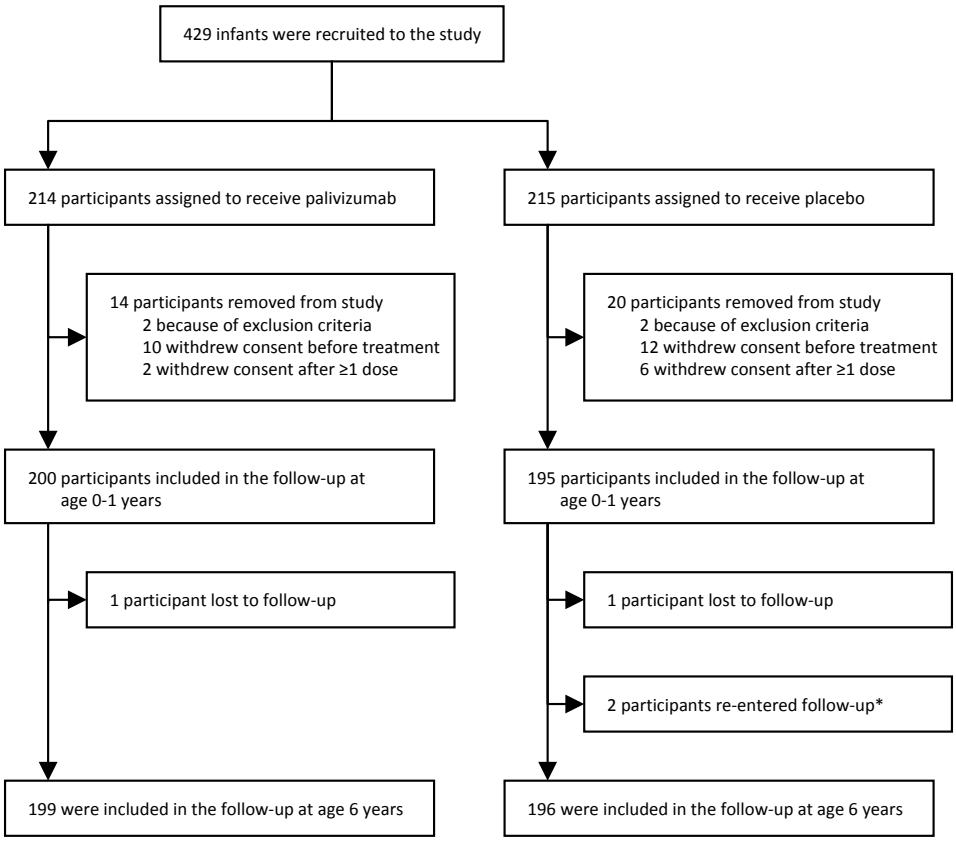

Figure 1. Study population flow chart
*Both children who re-entered the study had received placebo and their parents withdrew consent after one and three injections respectively, but gave consent for all other study assessments.

23 participants in the RSV prevention group and 26 (70.3%) of 37 participants in the placebo group reported that the wheeze was infrequent (one to three reported episodes in the past year). $FEV_{0.5}$ percentage predicted values were similar between the RSV prevention group (89.1 [SD 10.6%]) and placebo group (90.1 [11.1%]), although less than 100% of predicted (Table 1, Figure 2). When multiple imputation was used to address incomplete data, primary outcome results were similar to those without imputation (appendix).

At follow-up at age 3 years, 36 (19.7%) of 183 children in the RSV prevention group had had wheeze since their first birthday, compared with 54 (28.9%) of 187 children in the placebo group (ARR 9.2% [95% CI 0.5-17.9]; Table 2). The proportion

Table 1. Current asthma and $FEV_{0.5}$ at age 6 years according to treatment group

	RSV prevention (n = 199)	Placebo (n = 196)	Absolute risk reduction (95% CI)	Relative risk reduction (95% CI)	Mean difference (95% CI)
Current asthma	28 (14.1%)	47 (24.0%)	9.9 (2.2 to 17.6)	41.3 (10.3 to 61.6)	
$FEV_{0.5}$ percentage predicted*	89.1 (10.6)	90.1 (11.1)			1.0 (-1.3 to 3.3)

Data are n (%) or mean (SD). Absolute and relative risk reduction given in percentage points. RSV = respiratory syncytial virus. $FEV_{0.5}$ = forced expiratory volume in 0.5 s. *Data available for 342 children.

of children who developed wheeze after the first year of life was low and similar between children receiving RSV prevention (12 [6.0%] of 199) and placebo (13 [6.6%] of 196; data not shown).

Data from physicians showed a similar frequency of physician-diagnosed asthma in the past 12 months between the groups (19 [10.3%] of 185 in the RSV prevention group vs. 18 [9.9%] of 182 in the placebo group). No difference was found between the proportions of children with eczema, allergic rhinitis, or food allergy in the RSV prevention and placebo group (Table 2). Children in the RSV prevention group had lower median total IgE values (45.0 kU per litre [IQR 18.0-136.0])

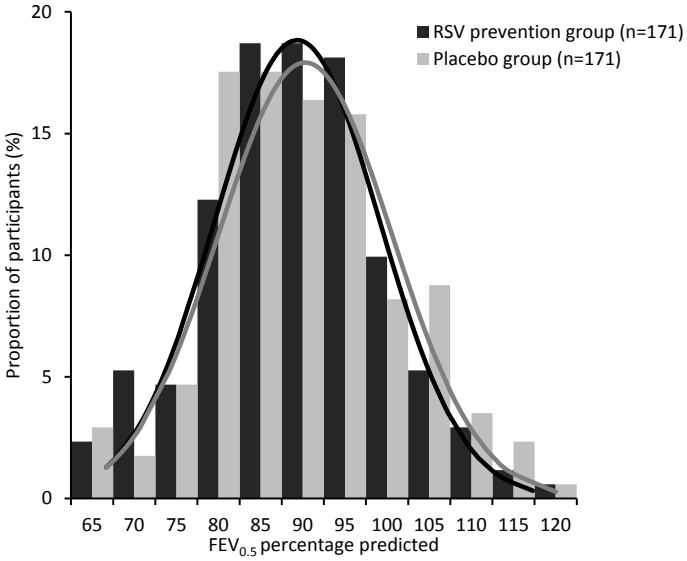

Figure 2. $FEV_{0.5}$ percentage predicted at age 6 years
$FEV_{0.5}$ = forced expiratory volume in 0.5 seconds. RSV = respiratory syncytial virus.

Table 2. Secondary outcome results

	RSV prevention	Placebo	Absolute risk reduction (95% CI)	Relative risk reduction (95% CI)
Follow-up at age 3 years				
Wheeze at ages 1-3 years	36/183 (19.7%)	54/187 (28.9%)	9.2 (0.5 to 17.9)	31.9 (1.5 to 52.9)
Current use of asthma medication	14/184 (7.6%)	23/188 (12.2%)	4.6 (-1.4 to 10.7)	37.8 (-17.1 to 67.0)
Follow-up at age 6 years				
Wheeze in the past 12 months*	23/199 (11.6%)	39/196 (19.9%)	8.3 (1.2 to 15.5)	41.9 (6.5 to 63.9)
Infrequent wheeze (1-3 episodes)	12/199 (6.0%)	26/194 (13.4%)	7.4 (1.5 to 13.2)	55.0 (13.4 to 76.6)
Frequent wheeze (>3 episodes)	11/199 (5.5%)	11/194 (5.7%)	0.1 (-4.4 to 4.7)	2.5 (-119.6 to 56.7)
Use of asthma medication in the past 12 months*	18/199 (9.0%)	25/195 (12.8%)	3.8 (-2.4 to 9.9)	29.5 (-25.1 to 60.2)
Nocturnal cough in the past 12 months	53/199 (26.6%)	57/195 (29.2%)	2.6 (-6.3 to 11.5)	8.9 (-25.2 to 33.7)
Use of oral corticosteroids for respiratory symptoms ever	4/199 (2.0%)	8/195 (4.1%)	2.1 (-1.3 to 5.5)	51.0 (-60.1 to 85.0)
Medically attended respiratory infection in the past 12 months	87/199 (43.7%)	83/195 (42.6%)	-1.2 (-10.9 to 8.6)	-2.7 (-28.9 to 18.1)
Eczema	68/199 (34.2%)	66/195 (33.8%)	-0.3 (-9.7 to 9.0)	-1.0 (-32.9 to 23.3)
Allergic rhinitis	22/199 (11.1%)	17/195 (8.7%)	-2.3 (-8.2 to 3.5)	-26.8 (-131.4 to 30.5)
Food allergy	20/199 (10.1%)	14/195 (7.2%)	-2.9 (-8.4 to 2.7)	-40.0 (-169.2 to 27.2)
Physician-diagnosed asthma in the past 12 months	19/185 (10.3%)	18/182 (9.9%)	-0.4 (-6.5 to 5.8)	-3.8 (-91.4 to 43.7)
Total IgE, kU per L†	45.0 [18.0-136.0]	68.0 [28.5-182.5]‡		
Specific IgE ≥0.35 kU per L against any aeroallergen	38/139 (27.3%)	27/105 (25.7%)	-1.6 (-12.8 to 9.5)	-6.3 (-62.3 to 30.4)

Data are n/N (%) or median [IQR]. Absolute and relative risk reduction given in percentage points. RSV = respiratory syncytial virus. *Used for primary outcome. †Data available for 240 participants. ‡Between-group difference tested with Mann-Whitney U test; p = 0.04.

than children in the placebo group (68.0 kU per litre [28.5-182.5]; p = 0.04), but the proportions of children with allergic sensitisation to the specific aeroallergens were all similar between the two groups (appendix).

Lung function results were similar between the RSV prevention and placebo groups. As expected in this population of preterm infants, FEV_1, FEF_{25-75}, $FEV_{0.5}$:FVC ratio, and FEV_1:FVC ratio were less than 100% of predicted, but similar between the RSV prevention and placebo groups (Table 3). Reversibility expressed as change in percentage predicted $FEV_{0.5}$ or FEV_1, fraction of exhaled nitric oxide, and resistance of the respiratory system were similar between the RSV prevention and placebo groups. $FEV_{0.5}$ and other lung function results were also similar after administration of a bronchodilator ($FEV_{0.5}$ percentage predicted 97.2% and 97.6% in the RSV prevention and placebo group respectively).

Table 3. Secondary outcome lung function results at age 6 years

	Children with available data	RSV prevention	Placebo	Mean difference (95% CI)
FEV_1 percentage predicted	342	91.5 (9.3)	92.3 (9.8)	0.8 (-1.2 to 2.9)
FVC percentage predicted	342	97.9 (10.9)	98.2 (10.1)	0.4 (-1.9 to 2.6)
FEF_{25-75} percentage predicted	342	96.3 (22.7)	98.0 (22.9)	1.7 (-3.2 to 6.5)
$FEV_{0.5}$:FVC ratio percentage predicted	342	91.4 (10.3)	91.7 (9.3)	0.2 (-1.9 to 2.3)
FEV_1:FVC ratio percentage predicted	342	93.2 (7.2)	93.3 (6.4)	0.1 (-1.3 to 1.6)
$FEV_{0.5}$ percentage change after bronchodilator	290	8.1 (5.8)	7.6 (5.7)	-0.5 (-1.8 to 0.8)
FEV_1 percentage change after bronchodilator	290	4.8 (5.0)	4.3 (4.9)	-0.5 (-1.6 to 0.7)
Fraction of exhaled nitric oxide, ppb	306	8.0 [6.0-12.0]	7.0 [6.0-10.0]*	
Resistance of the respiratory system, kPa L^{-1} s	313	0.84 [0.74-0.97]	0.82 [0.70-0.95]†	

Data are mean (SD) or median [IQR]. RSV = respiratory syncytial virus. FVC = forced vital capacity. FEF_{25-75} = forced expiratory flow between 25% and 75% of vital capacity. $FEV_{0.5}$ = forced expiratory volume in 0.5 s. *Between-group difference tested with Mann-Whitney U test; p = 0.13. †Between-group difference tested with Mann-Whitney U test; p = 0.33.

In the post-hoc subgroup analysis of children with and without atopy, the prevalence of parent-reported current asthma at age 6 years was higher in the placebo group than the RSV prevention group regardless of atopic status, although this difference was not significant for all statuses (appendix). Stratification by follow-up study year (2014-16) or location of study measurements (research centre vs. home visit) did not change primary outcome results (data not shown). None of the post-hoc analyses revealed subgroup-specific lung function differences between children in the RSV prevention and placebo group. Analysis of lung function showed that mean $FEV_{0.5}$ percentage predicted was lower in the 65 children with parent-reported current asthma (86.1% [SD 12.1]) than in the 277 children without current asthma (90.4% [10.4]; mean difference 4.2 [95% CI 1.3 to 7.2]); appendix). Similarly, $FEV_{0.5}$ percentage predicted was lower in the 32 children with current physician-diagnosed asthma (85.1% [SD 15.0]) than in the 288 without (90.0% [10.4]; mean difference 4.9 [95% CI -0.6 to 10.4]). Current asthma at age 6 years was slightly more prevalent in children with RSV-related hospital admission during the first year of life (five [45.5%] of 11 children) than in children who were not admitted to hospital (70 [18.2%] of 384; ARR 27.2% [95% CI -2.5 to 56.9]); whereas $FEV_{0.5}$ percentage predicted at age 6 years was slightly lower in patients who had been admitted to hospital (n = 10; 85.5% [SD 15.4]) than in those who had not (n = 332; 89.7% [10.7]; mean difference 4.2% [95% CI -6.8 to 15.2]; data not shown).

Discussion

In this RSV prevention trial assessing the causal link between RSV infection and asthma, we found that RSV prevention in otherwise healthy preterm infants reduced the risk of parent-reported asthma at age 6 years, which was mostly explained by differences in infrequent wheeze. Furthermore, at age 6 years, no difference was noted in physician-diagnosed asthma in the past year or in lung function.

Only a few asthma prevention trials have been able to follow children up to an age at which pulmonary function testing is possible.[24–27] Most studies investigated inhaled or systemic glucocorticosteroids or mast cell stabilisers in high-risk populations. MAKI is the first randomised RSV prevention trial with pulmonary function testing in participants of any age. We showed that RSV prevention was associated with a decrease in parent-reported wheeze at 6 years of age. Similar results have been reported by others,[28] including a case-control study from Japan showing that palivizumab prophylaxis during infancy was associated with a reduction of parent-reported recurrent wheeze up to age 6 years.[7] However, our study showed no effect of RSV prevention on current physician-diagnosed asthma at age 6 years. A randomised controlled trial in term native American infants using motavizumab for RSV prophylaxis also showed no effect of RSV prevention on medically attended wheeze up to age 3 years.[15,29,30] Two possible explanations exist for the differences we observed between parent-reported and physician-diagnosed asthma. First, information bias (reporter) might have occurred because parents were informed of treatment allocation, and because the occurrence of asthma symptoms earlier in life might have influenced recognition and reporting of asthma at age 6 years. Second, asthma symptoms might have not been severe enough to warrant a doctor's visit. Altogether, this has led us to conclude that RSV prevention does not reduce clinically relevant asthma symptoms at age 6 years.

Overall spirometry results showed decreased lung function compared with reference values, which is in line with previous studies showing that childhood lung function is reduced in preterm children, even those who are only mildly preterm and otherwise healthy.[28,31,32] Although the correlation between asthma and lung function is generally poor,[33] other studies have shown reduced lung function values in children with asthma,[34–36] which is in line with the lower $FEV_{0.5}$ values seen in children with current asthma in our trial regardless of treatment group.

Our study has some strengths and limitations. The major strengths of our study are the randomised design, which precludes bias from selection or confounding, the proportion of trial participants who responded, and the lung function testing. Some potential limitations should also be discussed. First, follow-up of study participants occurred single-blind after the first year of life and might have resulted in reporting bias

by parents. Second, we used a patient-reported outcome as recommended for RSV prevention trials during a recent FDA-NIH workshop, which might have been sensitive to reporting bias by parents as well.[19] For example, the presence or absence of asthma symptoms during infancy might have influenced recognition and the threshold of labelling respiratory symptoms as wheeze or asthma in later childhood. Together, this might have led to an overestimation or underestimation of asthma prevalence. We aimed to minimise inconsistent reporting by having the same team of researchers who were unaware of treatment allocation taking the questionnaire and by studying objective outcomes such as lung function. Third, this trial was conducted in healthy preterm infants in a high-income setting and our results cannot be generalised to term infants or to lower-income regions. Fourth, our study has not been powered to detect smaller, but clinically relevant effects of RSV prevention on different asthma phenotypes. Fifth, our study might be hampered by missing lung function data, which might have resulted in reduced power to detect differences in lung function, especially for subgroup analyses. However, the proportion of successful lung function data (87%) in our study was similar to what was observed in other trials (83-84%).[37,38] Sixth, lung function was assessed only when children were free of respiratory symptoms and during spring or summer, which might have resulted in an underestimation of lung function deficits. The use of a bronchial challenge test might have increased sensitivity to detect airway reversibility, but was considered infeasible in our cohort of young children. Seventh, the prevalence of current physician-diagnosed asthma in this study might be underestimated; physician-attended asthma symptoms in children who attended another physician or hospital than mentioned by parents could have been missed. However, information about these medical events is usually reported to the patient's general practitioner in the Netherlands. Eighth, RSV prevention by other immunisation strategies than palivizumab immunoprophylaxis, such as maternal vaccination or active immunisation by a child vaccine, might have different effects on subsequent wheeze and asthma development. Results of this study therefore cannot be extrapolated to the general population. Ninth, our study has not been designed to measure cost-effectiveness of RSV prevention and results are therefore not directly applicable to cost-effectiveness analyses of future RSV preventive strategies. Finally, we did not account for multiple testing with regard to the current asthma and lung function outcomes. Bonferroni correction would not have changed our results.

Reflection on lessons learnt from this asthma prevention trial shows the essential value of objective outcome measures. We would therefore consider combining subjective outcomes, such as physician-confirmed asthma symptoms and patient-reported outcomes, with objective lung function measurements, which can only be measured reliably in children aged at least 6 years.

In conclusion, our study was designed to establish the causal relationship between RSV infection during infancy and asthma up to the age of 6 years in otherwise healthy preterm infants. RSV prevention did not have a major effect on current asthma or lung function at age 6 years. RSV prevention only reduced the risk of parent-reported infrequent wheeze at age 6 years, while the risk of physician-diagnosed asthma did not decrease. Future RSV vaccine studies will inform on whether RSV prevention is related to the risk of asthma at school age in the general population.

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Appendix

Supplementary material

Detailed description of lung function methods

The second primary outcome was $FEV_{0.5}$ assessed by forced spirometry. $FEV_{0.5}$ was chosen as primary endpoint instead of forced expiratory volume in 1 second (FEV_1) because of the young age of study participants.[1] Secondary outcomes were FEV_1, forced vital capacity (FVC), forced expiratory flow between 25% and 75% of VC (FEF_{25-75}), $FEV_{0.5}$:FVC ratio, and FEV_1:FVC ratio. All respiratory function assessments were performed according to the ATS/ERS guidelines.[1] If the child suffered from a respiratory tract infection or was required to use salbutamol for symptoms, the study visit was rescheduled. To minimize lung function variability due to concurrent respiratory tract infections all study visits were scheduled during spring or summer. Spirometry was performed using a calibrated spirometer (ZAN 100 hand-held pulmonary spirometer system, nSpire, USA). The best values of $FEV_{0.5}$, FEV_1, FVC, FEF_{25-75}, and other parameters were selected from correctly performed manoeuvres. Spirometry results were converted into sex-, age-, and height-adjusted percent-predicted values using Dutch normative values.[2] Reversibility was assessed by repeated spirometry 15 minutes after administration of 800 microgram salbutamol (8 puffs) via metered-dose inhaler and valve spacer. Differences in $FEV_{0.5}$ and FEV_1 after administration of salbutamol were expressed as absolute change in percent-predicted value (post minus pre value). In addition to spirometry, the fraction of exhaled nitric oxide (FeNO) was measured in exhaled breath (NIOX MINO and NIOX Vero, Aerocrine AB, Solna, Sweden). The resistance of the respiratory system (Rint) was assessed by use of the interrupter technique (MicroRint, Micro Medical Limited, Kent, United Kingdom). Median Rint was calculated from at least eight acceptable interruptions.

Detailed description of statistical analysis

All analyses were performed on an intention to treat basis. The primary and secondary outcomes were pre-specified (see Statistical analysis plan in the Supplementary Appendix). Current physician-diagnosed asthma was defined post-hoc. Post hoc subgroup analyses were done to assess the influence of atopic status, intertester variability, differences over study duration (2014-2016), and study site (home visit or research centre). The influence of current asthma status on lung function was evaluated as well. Percentages and associated 95% confidence intervals were calculated for the prevalence of dichotomous outcomes, whereas mean or median values were

calculated for continuous outcomes depending on normality distribution. Study groups were compared by calculating absolute risk reductions (ARR), relative risk reductions, or mean differences with 95% confidence interval (CI). If applicable, between-group differences were assessed using Chi-squared, Fisher's exact, t-test or Mann-Whitney U statistical tests. MAKI was powered to determine the effect of RSV prevention on total number of wheezing days during the first year of life.[3] For the current study, the same study population was conveniently used. Therefore, we provide the differences we could prove assuming an alpha of 5% and a power of 80%. For asthma, 395 patients were included, which means that we could prove a clinically relevant difference of 9% or more based on the asthma prevalence of 24% in the placebo group of our trial. For lung function measurement, 342 patients were included, which means that we could prove a clinically relevant difference of 7% or more based on the $FEV_{0.5}$ percent-predicted of 90.1% in the placebo group of our trial. Complete case analysis was done if less than 10% of data were missing. Otherwise, we studied the impact of the missing data by performing multiple imputation and comparing the results of the complete case analysis with those from the multiple imputation. Approximately 100 baseline and outcome variables at age 1, 3 and 6 years were selected to be part of the imputation model and covered RSV-related outcomes during the first year of life, parental atopy and smoking, wheeze and asthma symptoms, child atopy and lung function. Statistical analyses were done with SPSS statistical software, version 22.0, except for multiple imputation, which was done with R, version 3.1.1 using the package mice, which makes an imputation model for each missing variable.[4] Diagnostic convergence plots were checked to observe the quality of the imputations.

Supplemental Table 1. Clinical characteristics at 6 years of age

	RSV prevention (n = 199)	Placebo (n = 196)
Age, years	5.8 (0.3)	5.9 (0.3)
Height, cm (range)	116.3 (105.4-131.0)	116.1 (103.7-129.0)
Weight, kg (range)	21.1 (14.7-36.8)	20.7 (14.6-32.2)
Parental smoking		
Father	53/192 (27.6%)	50/193 (25.9%)
Mother	35/198 (17.7%)	37/194 (19.1%)
Inside home	11/199 (5.5%)	17/195 (8.7%)

Data are n/N (%) or mean (SD). RSV = respiratory syncytial virus. Height and weight data were available for 364 children.

Supplemental Table 2. Primary results after multiple imputation

	Children with available data	RSV prevention	Placebo	Absolute risk reduction (95% CI)	Relative risk reduction (95% CI)	Mean difference (95% CI)
Current asthma	429	35.6 (16.6%)	57.3 (26.7%)	10.0 (1.9 to 18.1)	37.6 (7.9 to 57.7)	
$FEV_{0.5}$ percentage predicted	429	88.6 (10.4)	89.7 (11.1)			-1.1 (-3.2 to 1.0)

Data are n (%) or mean (SD). Absolute and relative risk reduction given in percentage points. RSV = respiratory syncytial virus. $FEV_{0.5}$ = forced expiratory volume in 0.5 s.

Supplemental Table 3. Additional results at 6 years of age according to treatment group

	Children with available data	RSV prevention	Placebo	Absolute risk reduction (95% CI)	Relative risk reduction (95% CI)
Medically attended otitis in the past 12 months	394	47 (23.6%)	52 (26.7%)	3.0 (-5.5 to 11.6)	11.4 (-24.6 to 37.1)
Medically attended upper respiratory tract infection in the past 12 months	394	53 (26.6%)	51 (26.2%)	-0.5 (-9.2 to 8.2)	-1.8 (-41.6 to 26.8)
Medically attended pharyngitis in the past 12 months	394	25 (12.6%)	23 (11.8%)	-0.8 (-7.2 to 5.7)	-6.5 (-81.1 to 37.4)
Medically attended pseudocroup in the past 12 months	394	12 (6.0%)	7 (3.6%)	-2.4 (-6.7 to 1.8)	-68.0 (-317.7 to 32.5)
Medically attended lower respiratory tract infection in the past 12 months	394	21 (10.6%)	26 (13.3%)	2.8 (-3.6 to 9.2)	20.9 (-35.8 to 53.9)
Rhinitis symptoms with itchy or watery eyes in past 12 months	394	19 (9.5%)	16 (8.2%)	-1.3 (-7.0 to 4.3)	-16.4 (-119.6 to 38.3)
Eczema symptoms in past 12 months	393	29 (14.6%)	39 (20.0%)	5.4 (-2.1 to 12.8)	26.8 (-13.5 to 52.7)
Exercise induced wheeze in past 12 months	394	13 (6.5%)	12 (6.2%)	-0.4 (-5.2 to 4.4)	-6.2 (-126.8 to 50.3)
Respiratory infection induced wheeze in past 12 months	394	24 (12.1%)	38 (19.5%)	7.4 (0.3 to 14.6)	38.1 (0.9 to 61.4)
Exercise induced cough in past 12 months	381	12 (6.2%)	20 (10.6%)	4.4 (-1.2 to 10.0)	41.6 (-16.2 to 70.6)
Bronchodilator use in past 3 months	394	18 (9.0%)	21 (10.8%)	1.7 (-4.2 to 7.6)	16.0 (-52.7 to 53.8)
Inhaled corticosteroids use in past 12 months	383	8 (4.1%)	16 (8.5%)	4.3 (-0.5 to 9.2)	51.3 (-11.1 to 78.7)
Specific IgE ≥0.35 kU per L against house dust mite	244	24 (17.3%)	19 (18.1%)	0.8 (-8.9 to 10.5)	4.6 (-64.7 to 44.7)
Specific IgE ≥0.35 kU per L against birch	242	11 (8.0%)	10 (9.5%)	1.5 (-5.7 to 8.7)	15.7 (-91.0 to 62.8)
Specific IgE ≥0.35 kU per L against mugwort	242	4 (2.9%)	3 (2.9%)	-0.1 (-4.3 to 4.2)	NA
Specific IgE ≥0.35 kU per L against timothy	242	13 (9.5%)	14 (13.3%)	3.8 (-4.3 to 12.0)	28.8 (-44.9 to 65.0)
Specific IgE ≥0.35 kU per L against *Aspergillus fumigatus*	242	5 (3.6%)	0 (0.0%)	-3.6 (-6.8 to -0.5)	NA
Specific IgE ≥0.35 kU per L against dog	244	8 (5.8%)	5 (4.8%)	-1.0 (-6.6 to 4.6)	-20.9 (-258.9 to 59.3)
Specific IgE ≥0.35 kU per L against cat	244	14 (10.1%)	6 (5.7%)	-4.4 (-11.1 to 2.3)	-76.3 (-343.3 to 29.9)

Data are n (%). Absolute and relative risk reduction given in percentage points. RSV = respiratory syncytial virus. NA = not applicable or not calculable.

Supplemental Table 4. Current asthma and lung function at 6 years of age for various atopic outcomes

	Atopic status	Children with available data	RSV prevention	Placebo	Absolute risk reduction (95% CI)	Relative risk reduction (95% CI)	Mean difference (95% CI)
Current asthma	Atopic parents	245	20/125 (16.0%)	31/120 (25.8%)	9.8 (-0.3 to 20.0)	38.1 (-2.5 to 62.6)	
	No atopic parents	144	6/71 (8.5%)	15/73 (20.5%)	12.1 (0.8 to 23.4)	58.9 (-0.0 to 83.1)	
	Allergic sensitization	65	9/38 (23.7%)	11/27 (40.7%)	17.1 (-5.9 to 40.0)	41.9 (-20.6 to 72.0)	
	No allergic sensitization	179	8/101 (7.9%)	16/78 (20.5%)	12.6 (2.2 to 23.0)	61.4 (14.4 to 82.6)	
	Eczema	134	14/68 (20.6%)	20/66 (30.3%)	9.7 (-5.0 to 24.4)	32.1 (-23.0 to 62.5)	
	No eczema	260	14/131 (10.7%)	26/129 (20.2%)	9.5 (0.8 to 18.2)	47.0 (3.1 to 71.0)	
	Allergic rhinitis	39	9/22 (40.9%)	9/17 (52.9%)	12.0 (-19.4 to 43.4)	22.7 (-51.5 to 60.6)	
	No allergic rhinitis	355	19/177 (10.7%)	37/178 (20.8%)	10.1 (2.5 to 17.6)	48.4 (13.8 to 69.1)	
$FEV_{0.5}$ percentage predicted	Atopic parents	209	88.1 (10.6)	88.8 (11.5)			0.7 (-2.3 to 3.7)
	No atopic parents	128	90.7 (10.3)	92.3 (10.3)			1.5 (-2.1 to 5.2)
	Allergic sensitization	63	87.6 (11.3)	88.2 (13.0)			0.6 (-5.6 to 6.8)
	No allergic sensitization	171	89.1 (10.2)	89.6 (10.8)			0.6 (-2.6 to 3.7)
	Eczema	116	90.7 (11.1)	89.9 (11.9)			-0.8 (-5.0 to 3.4)
	No eczema	226	88.3 (10.3)	90.2 (10.8)			2.0 (-0.8 to 4.7)
	Allergic rhinitis	34	91.4 (13.3)	87.7 (11.8)			-3.7 (-12.7 to 5.3)
	No allergic rhinitis	308	88.8 (10.2)	90.3 (11.1)			1.6 (-0.8 to 4.0)

Data are n/N (%) or mean (SD). Absolute and relative risk reduction given in percentage points. RSV = respiratory syncytial virus. $FEV_{0.5}$ = forced expiratory volume in 0.5 s.

Supplemental Table 5. Lung function at 6 years of age according to current asthma status

	Treatment group	Children with available data	Current asthma	No current asthma	Mean difference (95% CI)
$FEV_{0.5}$ percentage predicted	Total population	342	86.1 (12.1); n = 65	90.4 (10.4); n = 277	4.2 (1.3 to 7.2)
	RSV prevention	171	85.5 (13.0); n = 23	89.6 (10.1); n = 148	4.1 (-0.5 to 8.8)
	Placebo	171	86.5 (11.8); n = 42	91.3 (10.7); n = 129	4.8 (0.9 to 8.6)

Data are mean (SD). RSV = respiratory syncytial virus. $FEV_{0.5}$ = forced expiratory volume in 0.5 s.

Supplemental Table 6. Lung function at 6 years of age according to frequency of wheeze

	Treatment group	Children with available data	Infrequent wheeze (1-3 episodes)	Frequent wheeze (>3 episodes)	Mean difference (95% CI)
$FEV_{0.5}$ percentage predicted	Total population	342	84.6 (10.9); n = 36	88.3 (16.2); n = 17	-3.7 (-12.7 to 5.3)
	RSV prevention	171	83.6 (12.8); n = 11	89.5 (16.0); n = 8	-5.9 (-19.8 to 8.0)
	Placebo	171	85.1 (10.2); n = 25	87.3 (17.4); n = 9	-2.2 (-15.9 to 11.4)

Data are mean (SD). RSV = respiratory syncytial virus. $FEV_{0.5}$ = forced expiratory volume in 0.5 s.

Statistical analysis plan at 6-year follow-up

Date: April 3, 2017

1. Study design and objective

The MAKI trial is a placebo-controlled RCT on the effect of RSV prevention by palivizumab on long term respiratory morbidity in late preterm infants. First results covered a one-year follow-up period and have already been published.[3] The single blinded follow-up of the same cohort of children at age six constitutes the current study. Researchers are blinded for treatment allocation (palivizumab vs. placebo administration during the first encountered RSV epidemic). The outcome of interest at follow-up at age six is the prevalence of current asthma and asthma-related outcomes.

2. Research question

The main research question is: Does RSV prevention by palivizumab administration early in life prevent asthma development at age six?

This is studied by the following sub questions:

- Does the prevalence of current asthma differ between the placebo and palivizumab groups at age six?
- Do lung function measurements differ between the placebo and palivizumab groups at age six?

3. Primary outcomes

Primary outcomes are:

- Current asthma defined as: parent-reported wheeze in the past 12 months and/or parent-reported use of asthma medication in the past 12 months
- Baseline $FEV_{0.5}$ (% predicted)

4. Secondary outcomes

Age 3

Questionnaire based outcomes

Parent-reported wheeze after the child's first birthday

Current use of airway medication

Age 6

Questionnaire based outcomes

Parent-reported wheeze in the past 12 months

Parent-reported physician diagnosed asthma ever
Parent-reported nocturnal cough in the past 12 months
Parent-reported use of asthma medication in past 12 months
Parent-reported eczema
Parent-reported allergic rhinitis
Parent-reported food allergy
Parent-reported medical attendance in the past 12 months for one of the following conditions: otitis, upper respiratory tract infection, pharyngitis, pseudocroup, lower respiratory tract infection.
Parent-reported use of oral corticosteroids ever for respiratory symptoms

Lung function based outcomes
FEV_1
Bronchodilator response based on $FEV_{0.5}$ and FEV_1 before and after administration of a bronchodilator
FVC
$FEV_{0.5}$/FVC
FEV_1/FVC
MEF_{25-75}
Bronchial NO (ppb)
Interrupter airway resistance (Rint)

Laboratory based outcomes
Atopy defined as: positive Phadiatop test result (specific IgE concentration 0.35 kU/L or above) using screening inhalation allergens
Total IgE

5. Data characteristics and definitions
During the first year of life data were collected by daily logs and at age one year ''any wheeze during the first year of life'' was defined as: parent-reported wheeze at least once among the available daily log data. At age three, symptom data were collected by a trial specific questionnaire containing a question on the presence of wheeze symptoms after the child's first birthday and on the current use of asthma medication. At age six, questionnaire based outcomes are collected by the ISAAC questionnaire supplemented with some trial specific questions. The definition of current asthma is based on the presence of wheezing symptoms and use of asthma medication. In the Netherlands asthma diagnosis is considered reliable from age seven and above. Consequently, asthma is rarely diagnosed before this age regardless of symptoms.

All individual spirometry results are independently assessed for technical erformance and data quality by investigators NS and KE, while blinded or treatment allocation and questionnaire results. Technical acceptability is then discussed by the two investigators in order to reach consensus.
All spirometry results are assessed according to ERS/ATS guidelines and publications on spirometry in pre-school children.[1,5–9] Reproducibility criteria used are a max 5% difference between the highest and second highest attempt for FEV_1 and FVC. At least three reproducible attempts are strived for, but due to young age of study participants and limited applicability of this ''adult'' ATS/ERS criterion for reproducibility, two reproducible attempts are considered suitable too. Regional and international reference values are used to calculate % predicted and/or z-scores for the various spirometry results.[2,10] Results before and after administration of a bronchodilator are given as absolute volume change or % predicted change (new - old value). Currently, there is no ATS/ERS definition available of reversibility based on a bronchodilator response for $FEV_{0.5}$. Due to the young age of study participants FEV_1 is expected to be close to the FVC.[1] Therefore, $FEV_{0.5}$ is primary outcome of the MAKI trial. MAKI is unblinded after all outcome measurements have been cleaned and ascertained and the complete database has been locked.

6. Statistical analysis

All above mentioned primary and secondary outcomes are considered dependent variables. For data analyses the administration of either palivizumab or placebo during the first year of life is the independent (dichotomous) variable. Intention to treat analysis will be performed comparing study participants randomized for palivizumab with those randomized for placebo administration. The two groups will be compared by calculating the rate difference, relative risk reduction or mean difference with 95% confidence interval.

Dealing with missing data

Missing data may lead to reduced power of the study and may be a source of bias. There is no formal rule regarding the maximum number of missing values that could be acceptable. As with other trials, it cannot be excluded that missing data are not missing at random. For the MAKI trial complete case analysis will be performed if less than 10% of data are missing. Otherwise, a sensitivity analysis will be performed to examine the impact of missing data on our primary analysis by comparing complete case analysis and analysis following multiple imputation using all available variables.

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RSV prevention in infancy and asthma in later life - Authors' reply

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We thank Eric A F Simões, Andrew H Liu, Xavier Carbonell-Estrany, and William Dupont and colleagues for their valuable comments on our trial[1] published in *The Lancet Respiratory Medicine* describing the effect of respiratory syncytial virus (RSV) prevention in infancy, by use of the monoclonal antibody palivizumab, on asthma and lung function at age 6 years. Simões and Liu inquired whether RSV prophylaxis might prevent asthma development in a subgroup of infants at high risk of recurrent wheeze. We agree that a subgroup analysis might reveal benefit for RSV prevention in specific populations. In our study,[1] we showed that children with and without atopy benefited similarly from RSV prevention. We have since analysed the effect of RSV prevention in 62 children who were exposed to maternal tobacco smoke in utero compared with 317 children who were not. Although exposure to maternal tobacco smoke in utero increased the risk of parent-reported current asthma at age 6 years (17 of 62 children [27%] vs. 52 [16%] of 317 children; p = 0.04), the relative risk reduction from RSV prevention was similar in both groups (Table 1). Additionally, RSV prevention did not affect lung function in either group (Table 1).

As suggested by Simões and Liu, we did additional longitudinal subgroup analyses. 165 (42%) of the 395 children in our study had experienced infant wheeze.[2] We found that, irrespective of treatment group, current asthma at age 6 years was more frequent in children with infant wheeze (43 [26%] of 165) than in those without (32 [14%] of 230; p = 0.002). Indeed, RSV prevention led to larger absolute and relative risk reductions in children with infant wheeze than in those without (Table 1). However, RSV prevention did not affect lung function in either group (Table 1). Taken together, these analyses confirm the beneficial effect of RSV prevention on parent-reported asthmatic symptoms, but not on lung function in any specific subgroup. Therefore, our data do not support a major benefit for RSV prevention on asthma in specific high-risk populations.

Table 1. Effect of maternal smoking during pregnancy and infant wheeze on current asthma and lung function at age 6 years

	Children with available data	**RSV prevention**	**Placebo**	**Absolute risk reduction (95% CI)**	**Relative risk reduction (95% CI)**	**Mean difference (95% CI)**
Current asthma						
Maternal smoking	62	6/30 (20.0%)	11/32 (34.4%)	14.4 (-7.4 to 36.2)	41.8 (-37.6 to 75.4)	
No maternal smoking	317	20/163 (12.3%)	32/154 (20.8%)	8.5 (0.4 to 16.7)	41.0 (1.3 to 64.7)	
Infant wheeze	165	12/66 (18.2%)	31/99 (31.3%)	13.1 (0.1 to 26.2)	41.9 (-4.7 to 67.8)	
No infant wheeze	230	16/133 (12.0%)	16/97 (16.5%)	4.5 (-4.8 to 13.7)	27.1 (-38.5 to 61.6)	
$FEV_{0.5}$ percentage predicted						
Maternal smoking	51	86.1 (10.6)	86.1 (11.4)			0.0 (-6.2 to 6.2)
No maternal smoking	276	89.6 (10.7)	91.2 (11.1)			1.6 (-1.0 to 4.1)
Infant wheeze	153	86.9 (11.6)	88.3 (11.3)			1.4 (-2.3 to 5.1)
No infant wheeze	189	90.3 (9.8)	92.2 (10.7)			1.9 (-1.0 to 4.9)

Data are n/N (%) or mean (SD). Absolute and relative risk reduction given in percentage points. RSV = respiratory syncytial virus. $FEV_{0.5}$ = forced expiratory volume in 0.5 s.

We agree with Dupont and colleagues that MAKI was underpowered to rule out a causal association between RSV infection and asthma. Indeed, a modest effect could still be of utmost relevance for health economic evaluations of future RSV vaccines or immunoprophylaxis. The WHO Initiative for Vaccine Research will soon publish the required sample size for a study to show a modest benefit of RSV prevention on asthma development. Carbonell-Estrany argues that our conclusion that RSV prevention did not have a major effect on asthma development was based on the secondary endpoint of physician-diagnosed asthma. Our conclusion was not based on this endpoint but on the absence of a difference in our co-primary endpoint of lung function. Finally, not RSV infection per se, but severe RSV infection leading to hospital admission might be associated with asthma at school age. We agree that severity of disease could be a risk factor for the development of asthma after RSV infection. However, the MAKI results suggest that this association reflects a common predisposition - for example, impaired lung function at birth in otherwise healthy late preterm infants - rather than causality.

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5

General discussion

Summary of the main findings of this thesis

The aim of this thesis was to study three different aspects of the RSV disease burden in young children. Global RSV-related childhood mortality was the first aspect studied. Clinical characteristics including age at death of children with fatal RSV infection were unknown. In **chapter 2** individual patient data of 358 children who had died with RSV in hospitals across the world were described. It was found that at least 28% of children with RSV-related in-hospital death have comorbidities. The presence of comorbidity influences the age distribution of RSV-related in-hospital death, especially in high-income countries. Most children in low-income and middle-income countries are younger than six months at the time of RSV-related in-hospital death.

The second aspect consisted of the potential effect of maternal vaccination on life-threatening and fatal RSV infection during infancy. While in phase-3 of clinical development, the expected effect of maternal vaccination on RSV prevention was still unknown. In **chapter 3** a mathematical model was developed to describe different factors that influence maternal vaccine efficacy. By applying this mathematical model to data from three cohorts of patients it was predicted that maternal vaccination against RSV may substantially decrease the number of life-threatening RSV infections in infants. Children with comorbidities (based on age at life-threatening infection) or preterm birth are likely to benefit less from maternal vaccination against RSV than healthy term children.

The third aspect comprised the causal relationship between RSV infection and asthma development. In **chapter 4** single, assessor-blinded follow-up of the randomised, placebo-controlled MAKI trial showed that prevention of RSV infection in otherwise healthy late preterm infants reduces the risk of parent-reported asthma at age six years, which is mostly explained by differences in infrequent wheeze. RSV prevention does not affect parent-reported frequent wheeze, lung function or physician-diagnosed asthma at age six years. Altogether, this follow-up trial demonstrates that despite the association found between RSV infection and asthma in non-randomised studies, a causal relationship seems unlikely in otherwise healthy late preterm infants. However, the trial may have missed detection of smaller, but clinically relevant effects of RSV prevention on different asthma phenotypes as a result of sample size.

This chapter serves as a reflection on the most important lessons learnt from this thesis. Implications of the main findings as well as suggestions for future research are further discussed below.

Global RSV-related childhood mortality

Reducing under-five mortality has been one of the key global development goals. The current sustainable development goal is to reduce under-five mortality to 25 per 1000 live births or lower and neonatal mortality to 12 per 1000 live births or lower by 2030.[1] The under-five mortality rate has already substantially decreased from in 69.4 per 1000 livebirths (95% UI 67.2-71.8) in 2000 to 38.4 per 1000 livebirths (95% UI 34.5-43.1) in 2016[2], but efforts should still be made to meet the 2030 goals. In 2016, neonatal mortality contributed to about 40% of under-five mortality. In post-neonates, half of deaths were caused by lower respiratory infections, diarrhoeal diseases and malaria with lower respiratory infections causing 20.7% to 25.3% of deaths.[2]

Since RSV is a major cause of lower respiratory infection in young children, it is important to know the contribution of RSV to the global mortality burden. The GBD 2015 working group estimated that 5.2% (95% UI 2.9-8.6) of deaths due to lower respiratory infections were attributable to RSV in 2015.[3] Estimates of the global number of RSV-associated deaths, however, vary and heavily depend on the methods and data sources used. For example, one of the most important publications on global RSV-related mortality used extensive unpublished and published data to model the overall number of deaths, but still faced limited availability of well-documented RSV-related deaths that occurred out of hospital.[4,5] Estimates for the number of in-hospital RSV-related deaths in 2015 by this RSV GEN working group (59 600; uncertainty range 48 000-74 500)[5] also differed from estimates by the GBD 2015 working group (36 400; 95% UI 20 400-61 500).[3] Both examples reflect the challenges in estimating the RSV-associated mortality burden when limited data are available for the population of children who die with RSV.[6]

This thesis also demonstrates that high-quality data for RSV-related deaths are scarce, especially in lower income regions where most mortality occurs.[3,5] In the global case series study as described in **chapter 2**, only a third of mortality cases were from low-income or lower middle-income countries. Data for key clinical variables such as prematurity and comorbidity were often missing as were data for socioeconomic risk factors for mortality. Although the data described in **chapter 2** form the largest study on RSV-related mortality so far, this study once again illustrates the need for high-quality data from lower income regions.

Age at RSV-related death

Understanding the age distribution for fatal RSV infection is important to target future RSV preventive interventions. Among the children with RSV-related in-hospital death studied in **chapter 2**, the presence of comorbidities affected age at death. This was best illustrated for children with RSV-related death from high-income countries, who

had the highest prevalence of comorbidities. Age at death for children from high-income countries with comorbidities was higher than for those without.
The same pattern was seen for children from high-income countries with life-threatening RSV infection who required admission to a paediatric intensive care unit (PICU) and survived (**chapter 3**). Children with comorbidities were older at the time of PICU admission than healthy term and healthy preterm children. Children with comorbidities are known to have a prolonged risk for life-threatening RSV infection and RSV-related in-hospital death.[7–10] This likely reflects a generally increased risk for severe disease course regardless of the causing respiratory pathogen.

When studying the clinical characteristics of children with RSV-related in-hospital death in **chapter 2**, it was hypothesized that if RSV-related death in children from lower income countries was mainly attributable to limited access to intensive care, the clinical profile of these children would be similar to that of children with life-threatening RSV infection admitted to a PICU in high-income countries (**chapter 3**). Although the prevalence of comorbidities was indeed similar in the two populations, children with RSV-related death in lower income countries were older than children admitted to a PICU in high-income countries. The difference between age at death and PICU admission could not be easily explained by disease duration or length of stay in hospital, as the time between onset of symptoms and RSV-related death in children from lower income countries was only ten days and length of stay in hospital was generally short.
We can only hypothesise about explanations for the age differences found. First, it could be the result of bias as both the global mortality cohort and PICU cohorts were sensitive to selection bias. For example, in lower income countries the youngest infants could already have died outside of hospital and thereby have driven the age distribution for children with in-hospital death. Second, several biological factors in children from lower income regions could play a role such as the prevalence of bacterial superinfection, undiagnosed comorbidity, or concomitant illness like malnutrition and immunodeficiency increasing the risk for severe disease course.[11–13] Third, sociodemographic factors like maternal educational level, indoor air pollution, access to care, availability of antibiotics and vaccine coverage could contribute to prolonged risk for RSV-related in-hospital death.[14,15]

Knowledge gaps
In addition to the scarcity of high-quality data for RSV-related mortality in lower income regions as described above, several gaps in knowledge about global RSV-related childhood mortality can be identified. First, it remains challenging to conclude on causality at the pathogen level. Although it is thought that RSV infection is generally

disease causing[16,17] (opposed to carrier state), the role of co-infection with other pathogens and of secondary bacterial infection in relation to mortality are unclear. In general, pathogen-attributable mortality estimates are sensitive to uncertainty as a result of clinically undistinguishable infections and co-incidence between pathogens.[18] Second, the contribution of RSV to neonatal mortality and non-respiratory classified mortality (e.g. sudden infant death, sepsis) is unknown. Third, age-specific mortality rates during the first months of life are not available. Fourth, the contribution of community deaths to the global RSV mortality burden remains uncertain. Initially 80% of lower respiratory infection related child deaths were estimated to occur outside of hospital[19], but more recent RSV estimates reported 26% to 50% of RSV-related child deaths to occur outside of hospital.[5,20]

One of the research initiatives launched to provide answers to some of these questions is the Child Health and Mortality Prevention Surveillance (CHAMPS) network, that aims to improve data quality for childhood mortality and uses post-mortem minimally invasive tissue sampling opposed to full autopsy.[6,21] Initiatives to improve quality of vital registries and verbal autopsy can contribute to improved understanding of childhood mortality as well. Research initiatives studying the respiratory disease and mortality burden should pay attention to case definitions inclusive for RSV bronchiolitis[22] and preferably include analysis of several pathogens, as clinical and socioeconomic profiles of children at increased risk for mortality are unlikely to be pathogen specific.[17,23]

RSV prevention

Different RSV immunisation strategies are currently under development[24,25] and three strategies for RSV prevention in young children are distinguished: maternal vaccination, paediatric vaccination and passive immunisation at birth with monoclonal antibody such as palivizumab. The findings of this thesis may have several implications for these future RSV immunisation strategies as discussed below.

Maternal vaccination model

Being one of WHO prioritised RSV prevention strategies[26], maternal vaccination against RSV is considered a promising public health intervention. The mathematical model described in **chapter 3** predicted that maternal vaccination may prevent a substantial proportion of global RSV-related in-hospital deaths and an even larger proportion of RSV-related PICU admissions in the United Kingdom and the Netherlands. However, certain knowledge gaps about maternal vaccination have been identified that influence the expected effect of maternal vaccination, as illustrated in our mathematical model (**chapter 3**).[27,28]

For example, the maternal vaccination model heavily depended on transplacental antibody transfer per week of gestation and on maternal vaccine characteristics such as vaccine-induced increase in maternal antibody level, antibody stability and maternal antibody decay in infants. Correlates of protection and the expected protective antibody threshold reflect other knowledge gaps.[27,28] The mathematical model further showed the effect of target population characteristics such as prematurity and age at RSV-related outcome on the potential of maternal vaccination. All three datasets used to apply the model to reflected retrospective patient cohorts and were sensitive to selection and reporting bias. Missing data for gestational age, comorbidity and age at outcome in days influenced the comparability of the cohorts and affected model output.

The maternal vaccination model could be improved by incorporating other factors that may influence antibody dynamics and vaccine efficacy such as reduced transplacental transfer, maternal immune response and RSV protective antibodies acquired by natural RSV infection during pregnancy. The potential effects of vaccine-induced protective antibodies in breastmilk and of varying protective antibody threshold should be accounted for as well. Complexity could be added by using this maternal vaccination model to develop a population level model for maternal vaccine effectiveness taking into account local vaccine coverage, RSV transmission patterns, risk of disease and disease severity, and the difference between delayed and prevented infection. The model should further be applied to different study populations in order to be able to predict the effect of maternal vaccination in the general population. Altogether, the maternal vaccination model illustrates the potential of maternal vaccination on prevention of life-threatening RSV infection and at the same time illustrates the uncertainty regarding the expected effects.

Window of protection

The desired protective window of RSV prevention is driven by age of children at the RSV-related outcomes that should be prevented. Age at the time of RSV-related PICU admission as described in **chapter 3** was in line with peak age at RSV-related hospitalisation in the United States, where most hospitalisations occurred during the second month of life and half of infant hospitalisations in the first three months of life.[29,30] As maternal vaccination is anticipated to provide protection during the first three months of life[31–33], similar to what is predicted in the maternal vaccination model in **chapter 3**, it seems likely that maternal vaccination could as well substantially reduce RSV-related hospital admissions in settings with similar age distribution data.

However, when considering age at RSV-related in-hospital death (**chapter 2 and 3**) maternal vaccination is predicted to be a less effective RSV prevention strategy. This

is in line with age younger than six months being a risk factor for RSV-associated hospitalisation[34] and with the estimate that 45% of global hospital admissions and in-hospital deaths occur in children younger than six months.[5] It is arguable that RSV prevention should provide protection during the first six months of life instead of first three and that maternal vaccination as single prevention strategy is insufficient to prevent life-threatening RSV infection.

Two alternative RSV immunisation strategies: paediatric vaccination (the second WHO prioritised RSV prevention strategy[26]) and passive immunisation at birth, could thus be considered (Figure 1). Maternal vaccination could be combined with paediatric vaccination at age three months to provide protection beyond the first three months of life, but a single preventive strategy providing protection during the first six months of life may actually be preferable in terms of feasibility, potential antibody interference and cost.[31] Passive immunisation with extended half-life monoclonal antibody administered right after birth is aimed to provide protection during the first five months of life[35,36] and could be a promising alternative. Additionally, it allows adaptation to high-risk infant populations, which is not possible for maternal vaccination.
Furthermore, interference of maternally derived antibody with vaccine response in infants could reduce vaccine efficacy and affect timing of a paediatric RSV vaccine and should be taken into account when combining different RSV preventive interventions. Paediatric vaccination should be timed relative to the clearance of passive antibody acquired through maternal vaccination or passive immunisation at birth, and the desired window of protection.

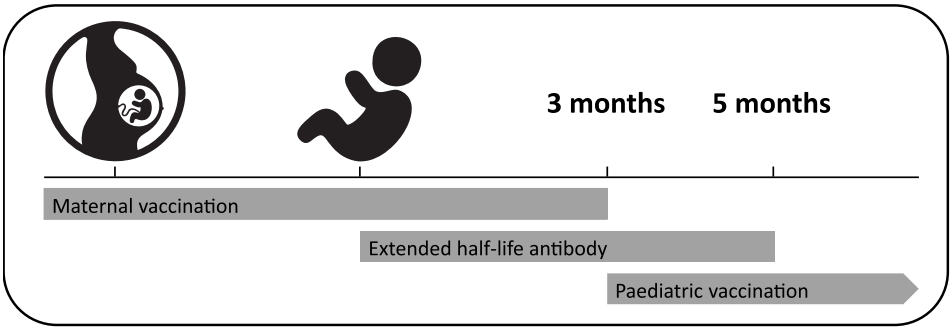

Figure 1. Expected duration of protection for three RSV immunisation strategies
Maternally-acquired antibodies will decrease after birth and the duration of protection is dependent on antibody levels at birth and antibody half-life thereafter. The expected duration of protection is up to three months.[37,38] Passive immunisation at birth with extended half-life antibody is expected to provide protection during five months.[35,36] Paediatric vaccination is generally possible from age two to three months and here assumed possible from age three months.

Timing of immunisation

The mathematical model (**chapter 3**) demonstrates the importance of adequate timing of maternal vaccine administration. Information about antenatal care utilisation by women in lower income regions is therefore required to estimate the potential of maternal vaccination as public health intervention in these regions. The same applies when considering passive immunisation with extended half-life monoclonal antibody at birth[35,36] as administration may be challenging in regions with limited access to new-born care and high prevalence of unattended home birth.

For both maternal vaccination and passive immunisation at birth, timing of administration further depends on RSV seasonality. In the United Kingdom, with a well-defined yearly RSV season, RSV prevention was predicted to be most cost-effective if only neonates born closely before the RSV season were protected.[31] In regions with unclear RSV seasonality, several incidence peaks or year-round activity, timing of maternal vaccination or passive immunisation at birth could thus be troublesome with regard to vaccine- and cost-effectiveness. Timing of immunisation should thus be adapted to target population characteristics, local healthcare policy and RSV seasonality.

Target population

Based on the findings of **chapter 2 and 3** maternal vaccination is unlikely to sufficiently protect certain high-risk populations such as preterm children and children with comorbidities from life-threatening and fatal RSV infection.

In preterm children, the potential of maternal vaccination heavily depends on transplacental antibody transfer, timing of vaccine administration and gestational age as illustrated in **chapter 3**. Since maternal vaccination is predicted to provide only short term protection (less than three months) in preterm children, they will require additional prevention against RSV. Additional passive immunisation at birth seems required, even if maternal vaccination is to be combined with paediatric vaccination at age three months. The preferred immunisation strategy will depend on future maternal vaccine efficacy, RSV disease risk in premature infants combined with the incidence of prematurity, potential indirect effects of immunisation (i.e. herd immunity), and on local public health policy. For example, more than 60% of global preterm births were estimated to occur in sub-Saharan Africa and South Asia in 2010.[39] The average global preterm birth rate was estimated to be 11.1% and nine out of 11 countries with rates of 15% or more were in sub-Saharan Africa.[39] The same regions are thought to face low perinatal healthcare utilisation. This means that if maternal vaccination is the preferred immunisation strategy, efforts should be made to increase new-born healthcare coverage to provide adequate care to preterm neonates in general and to provide additional RSV prevention after birth. Depending on feasibility and cost analysis,

one could argue that passive immunisation at birth could be preferred to maternal vaccination in these settings.
In children with comorbidities, extended protection against RSV is needed.[10] Both passive immunisation and paediatric vaccination could be considered in addition to or instead of maternal vaccination. With respect to the risk of RSV-related death in children with comorbidities (**chapter 2**), underlying vulnerability should be addressed as well especially in children from resource poor settings. In conclusion, the preferred RSV immunisation strategy in populations at high-risk for severe RSV infection may be different than in the general population.

Cost-effectiveness
Current passive immunisation with palivizumab is that expensive that administration is hard to achieve. A recent cost-effectiveness analysis found that the price of palivizumab needs to be reduced by 60% to become acceptable for a cost-effectiveness threshold of € 80 000 per QALY and by more than 90% to become cost-saving.[40] Short-term RSV-related outcomes such as infant hospitalisation mainly drive current cost-effectivity analyses. The reduction of childhood wheeze by RSV prevention could be taken into account when considering cost[41,42], but is inexpensive compared to hospitalisation.[43] If however, RSV prevention resulted in reduction of a chronic lung disease like asthma, it would more likely become cost-effective.[41] The results of the MAKI trial (**chapter 4**) make this scenario more unlikely and may therefore impact cost-effectiveness analysis of future RSV preventive interventions.

Future perspectives
This thesis confirms the global aspect of the RSV disease burden in children and demonstrates epidemiological differences between income regions, subgroup populations and RSV-related outcomes studied. In order to reduce the global RSV disease burden, RSV prevention strategies should be designed from a global perspective and be adapted to meet local aims, capacity and target populations. When discussing RSV prevention during the first six months of life, clear distinction should be made between immunisation providing protection during the first three months and six months and between the aimed prevented outcomes. Maternal vaccination may reduce substantial RSV disease burden in healthy term infants who form the absolute majority of RSV-related hospitalisations in high-income countries[30,44], but seems less effective in reducing global in-hospital mortality and in protecting high-risk populations such as children with prematurity or comorbidities. The two immunisation strategies prioritised by the WHO are unlikely to sufficiently reduce RSV-related life-threatening infections if aimed as single prevention strategy. The combination should be considered as should the option of extended half-life antibody administration at birth.

To further guide the development of preventive interventions, we need a better understanding of age-specific RSV incidence in term and preterm infants, the population attributable fractions of prematurity and comorbidity for the RSV disease burden, clinical and socioeconomic risk factors for RSV-related death in children from low-income and middle-income countries, expected maternal vaccine efficacy including transplacental antibody transfer and the protective threshold, and of expected costs of different immunisation strategies. High-quality prospective observational studies that are simultaneously done in different parts of the world would be required.

RSV and asthma

The relationship between RSV infection early in life and asthma development later in childhood has received much attention from RSV researchers, vaccine manufacturers and policy makers.[41,45–48] This relationship has been studied extensively in order to find out if RSV infection induces asthma development or if RSV disease and asthma are signs of a common susceptibility to paediatric lung disease. As described in **chapter 4** and in the brief summary above, the results of the MAKI trial showed that RSV prevention has no major effect on asthma development in otherwise healthy late preterm infants. The MAKI trial also demonstrates that conclusions on causality for a clinically heterogeneous disease like asthma can be challenging and that results of a randomised trial may be interpreted in various ways after all.

Asthma diagnosis

Several asthma phenotypes have been described and since definitions and diagnostic criteria for asthma vary, results from asthma research may be hard to interpret and compare.[49,50] For example, age at diagnosis influences the definition for asthma used and thus comparability of study results. Danish cohort studies on risk factors for asthma development have established asthma diagnosis at age three years[51,52], whereas Dutch clinical practice considers asthma diagnosis possible in children aged at least 6-7 years. The clinical phenotypes defined as asthma at age three and six years could differ substantially.

In the MAKI trial (**chapter 4**) asthma was defined as parent-reported wheeze symptoms in the past year and/or use of asthma medication in the past year. Other studies on asthma considered physician-diagnosed asthma required for asthma diagnosis in order to minimise subjectivity.[53,54] However, physician-diagnosed asthma contains elements of subjectivity as well since it is dependent on individual clinical practice of physicians, on healthcare seeking behaviour and reporting bias of patients, and on national considerations regarding asthma diagnosis in children. Physician-diagnosed

asthma is therefore valuable in addition to other subjective patient-reported asthma outcomes, but should not replace objective outcome measures required for asthma diagnosis such as lung function. In addition, in order to have added value for asthma diagnosis, physician-diagnosed asthma should preferably be based on data collected from physicians and medical records instead of on patient reports.[48]

Lung function

Although lung function measurements are considered standard objective outcomes for asthma diagnosis, the correlation with asthma symptoms can be poor.[55] The presence of airway reversibility at the time of asthma symptoms is confirmative for asthma diagnosis, but the absence of lung function deficits does not exclude asthma diagnosis and can be expected in well controlled or stable asthma patients. In the MAKI trial lung function was assessed in children free from respiratory symptoms and during summer, which together may have reduced sensitivity for asthma diagnosis. The use of a methacholine challenge test could have increased sensitivity to detect airway reversibility but was not considered feasible at the young age of study participants (5-6 years). To add complexity, criteria for airway reversibility in young children (expressed as change in $FEV_{0.5}$ and FEV_1) are actually not available.

Furthermore, spirometry criteria for technical performance, data interpretation and the use of reference data were adapted to the young age of study participants. $FEV_{0.5}$ was chosen primary outcome and values were converted to percentage predicted values with the use of normative values.[56] However, it is still challenging to obtain robust percentage predicted values for young children that are comparable between studies. For example, no reference value dataset contains both $FEV_{0.5}$ percentage predicted and z-value data[56,57] and available datasets generally contain limited data points for spirometry in this age group. As a result, the percentage predicted values for $FEV_{0.5}$ in our trial heavily depended on the exact number of decimal places used in the equations and should thus be interpreted with caution. As the trial aimed to examine between-group differences, this uncertainty did not affect main results.

Study design

Several lessons can be learnt from the MAKI trial for the design of future studies on childhood asthma and recommendations have been summarised in Figure 2. To minimise bias, unmasking of trial participants and researchers should be avoided before follow-up has been completed and both subjective and objective outcome measures should be studied. When trial design includes long-term follow-up of study participants, study outcomes and assessments should be predefined to maximise availability of similar data for study outcomes at different time points and to minimise the need for post-

hoc analyses. For example, our trial design could have been improved by inclusion of medically-attended outcomes and healthcare utilisation required for cost-effectiveness analyses and by describing these outcomes in the original trial protocol. Furthermore, sample size should allow detection of between-group differences during the course of the trial incorporating loss to follow-up of study participants when long-term follow-up (at least six years) is aimed for.

- Ensure blinding of researchers and parents until follow-up has been completed
- Use a universal, internationally recommended and age-specific asthma definition
- Record both patient-reported and physician-confirmed asthma symptoms
- Study objective outcomes in addition to subjective outcomes
- Clearly describe lung function procedures and interpretation
- Report on several lung function outcomes relevant for the age of trial participants
- Assess airway reversibility in children aged 5-6 years or older

Figure 2. Recommendations for follow-up childhood asthma trials

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6

Nederlandse samenvatting

Het respiratoir syncytieel virus (RSV) is een veelvoorkomende oorzaak van luchtweginfecties bij jonge kinderen. Vrijwel alle kinderen maken in de eerste twee levensjaren een RSV-infectie door. Een onderste luchtweginfectie veroorzaakt door RSV, typisch bronchiolitis, is een van de belangrijkste oorzaken van opname van jonge kinderen in het ziekenhuis. In Nederland en omliggende landen zorgt RSV jaarlijks voor een epidemie in de wintermaanden met een groot aantal ziekenhuisopnames op kinderafdelingen en intensive care afdelingen.
RSV-infecties komen niet alleen in Westerse landen voor, maar ook in landen met (sub) tropische klimaten. Wereldwijd veroorzaakte RSV in 2015 naar schatting 33,1 miljoen onderste luchtweginfecties bij kinderen jonger dan vijf jaar. In 10% van deze kinderen was de luchtweginfectie met RSV zo ernstig dat opname in het ziekenhuis noodzakelijk was. Hoewel in Nederland en andere rijke landen sterfte door een RSV-infectie zeldzaam is, is RSV wereldwijd gezien een belangrijke oorzaak van kindersterfte door onderste luchtweginfecties.
RSV-infecties veroorzaken niet alleen ziekteverschijnselen op korte termijn, maar kunnen ook gepaard gaan met klachten van benauwdheid op langere termijn. RSV-infecties worden daarnaast in verband gebracht met het optreden van astma, maar het is nog onduidelijk of een RSV-infectie en astma gewoon vaker samen voorkomen in mensen met daarvoor gevoelige longen of dat een RSV-infectie ook astma kan veroorzaken.
Er bestaat momenteel geen behandeling voor RSV-infectie, waardoor alleen ondersteunende behandeling mogelijk is in de vorm van zuurstof, vocht, voeding en ademhalingsondersteuning. Ook bestaat er geen vaccin om RSV-infectie te voorkomen. Tijdelijke bescherming door passieve immunisatie (het toedienen van beschermende antistoffen) met palivizumab, een gehumaniseerde monoklonale antistof, is vanwege de hoge kosten alleen beschikbaar in rijke landen en alleen geïndiceerd in specifieke hoog risicogroepen. Er zijn echter meerdere vormen van passieve en actieve immunisatie (vaccinatie) in ontwikkeling om RSV-infectie te voorkomen. Maternale vaccinatie, waarbij zwangere vrouwen worden gevaccineerd tegen RSV en vervolgens via de placenta beschermende antistoffen doorgeven aan het ongeboren kind, is bijvoorbeeld in een vergevorderd stadium van ontwikkeling. Maternale vaccinatie is een veelbelovende strategie om infectieziekten direct na de geboorte tot enkele maanden daarna te voorkomen.

Drie aspecten van de RSV-gerelateerde ziektelast in jonge kinderen vormden de basis voor het onderzoek dat is beschreven in de verschillende hoofdstukken van dit proefschrift.

Het eerste aspect was wereldwijde kindersterfte ten gevolge van RSV-infecties. Het doel van het onderzoek beschreven in **hoofdstuk 2** was om de kenmerken van kinderen die wereldwijd overlijden aan een RSV-infectie in kaart te brengen. Voor dit internationale onderzoek werden onderzoekers en artsen benaderd om klinische gegevens te delen over RSV-gerelateerde sterfgevallen. De individuele gegevens van 358 kinderen jonger dan vijf jaar met RSV-gerelateerde sterfte werden bestudeerd. De sterfgevallen waren afkomstig uit 23 verschillende landen en 33% van de sterfgevallen was afkomstig uit lagere inkomenslanden.
Het onderzoek liet zien dat in lage- en midden-inkomenslanden de meerderheid van de bestudeerde kinderen jonger dan zes maanden was op het moment van overlijden. Een aanzienlijk deel van de kinderen die waren overleden aan RSV-infectie had onderliggend lijden, zoals een aangeboren hartziekte of longziekte. In hoge-inkomenslanden had zelfs 70% van de overleden kinderen onderliggend lijden (ook wel co-morbiditeit genoemd). In hoge-inkomenslanden waren deze kinderen bovendien ouder op het moment van overlijden dan gezonde kinderen. Gegevens over welke kinderen te vroeg geboren (prematuur) waren en onderliggend lijden hadden, ontbraken echter regelmatig voor kinderen uit lage-inkomenslanden. Hetzelfde gold voor gegevens over sociaaleconomische kenmerken van deze kinderen.
Hieruit bleek dat het erg lastig is om voldoende gegevens te verkrijgen van kinderen die wereldwijd overlijden aan een RSV-infectie. Om kindersterfte gericht terug te kunnen dringen, is het echter belangrijk kennis te hebben van de kinderen die het meeste risico lopen. Daarnaast liet dit onderzoek zien dat, op basis van de leeftijd bij overlijden, maternale vaccinatie slechts beperkte bescherming zal bieden tegen sterfte door RSV-infectie in kinderen met onderliggend lijden. Deze kinderen zullen extra vormen van bescherming tegen RSV nodig hebben, zoals passieve immunisatie bij de geboorte of actieve immunisatie (kind vaccinatie) enkele maanden later.

Het tweede aspect en doel van dit proefschrift betrof het voorspellen van het effect van maternale vaccinatie op levensbedreigende en fatale RSV-infecties. Door middel van de ontwikkeling van een mathematisch model werden in **hoofdstuk 3** de verschillende factoren bestudeerd die de effectiviteit van een maternaal vaccin tegen RSV beïnvloeden. Onder andere de stijging van antistoffen bij de moeder na vaccinatie, het doorgeven van antistoffen via de placenta tijdens de zwangerschap, en de afname van antistoffen in de pasgeborene werden in het model meegenomen. Door dit model vervolgens toe te passen op drie groepen kinderen met levensbedreigende of fatale RSV-infecties tijdens het eerste levensjaar, kon worden berekend welk deel van deze RSV-infecties en sterfte zou kunnen worden voorkomen door maternale vaccinatie.
Het model liet zien dat maternale vaccinatie bij een zwangerschapsduur van 30 weken

62-87% van de levensbedreigende RSV-infecties en 29-84% van de RSV-gerelateerde sterfgevallen zou kunnen voorkomen. Volgens het model zou maternale vaccinatie aan te vroeg geboren kinderen en kinderen met onderliggend lijden minder bescherming kunnen bieden tegen levensbedreigende of fatale RSV-infecties dan aan gezonde, op tijd geboren kinderen. Te vroeg geboren kinderen en kinderen met onderliggend lijden dienen op basis van dit onderzoek dan ook extra te worden beschermd tegen RSV-infectie.
Het model liet daarnaast zien dat het voorspelde effect van maternale vaccinatie voor een belangrijk deel wordt bepaald door wanneer in de zwangerschap het vaccin wordt toegediend. Daarnaast bleek dat klinische gegevens van hoge kwaliteit over maternale vaccinkenmerken beperkt beschikbaar zijn, terwijl die de modeluitkomsten sterk beïnvloeden. Bijvoorbeeld de gekozen afnamesnelheid van antistoffen in de pasgeborene was in het model van grote invloed op het voorspelde effect van maternale vaccinatie.

Het derde aspect was de relatie tussen RSV-infectie en de ontwikkeling van astma. Astma is een van de meest voorkomende chronische ziekten wereldwijd. Kennis over mogelijke oorzaken, en daaruit volgend mogelijkheden voor preventie, van astma is daarom van belang. De vraag of RSV-infectie een oorzaak is van astma of dat RSV-infectie en astma beide het gevolg zijn van aanwezige gevoeligheid voor deze longziekten kan alleen worden beantwoord door een gerandomiseerde, placebo-gecontroleerde studie.
Zodoende is de MAKI studie opgezet om het effect van RSV-bescherming op het optreden van piepende ademhalingsklachten te onderzoeken en is deze studie gecontinueerd om het effect van RSV-bescherming op astma ontwikkeling te onderzoeken. In **hoofdstuk 4** is beschreven hoe 429 gezonde, te vroeg geboren kinderen tijdens het eerste levensjaar of palivizumab kregen ter preventie van RSV-infectie of een placebo kregen. Vervolgens is bij 395 van deze kinderen op de leeftijd van zes jaar onderzoek gedaan naar het optreden van astma en naar hun longfunctie.
Dit vervolgonderzoek liet zien dat alleen door ouders gerapporteerde, relatief milde piepende ademhalingsklachten werden verminderd door preventie van RSV-infectie. Preventie van RSV-infectie gaf geen vermindering in het gebruik van astmamedicatie of in astma gerapporteerd door de huisarts. Daarnaast had preventie van RSV-infectie geen effect op longfunctie op zesjarige leeftijd. Hiermee liet dit onderzoek zien dat in gezonde, te vroeg geboren kinderen het onwaarschijnlijk is dat RSV-infectie een klinisch relevante, oorzakelijke rol speelt in de ontwikkeling van astma. Een oorzakelijke rol in milde vormen van astma of in de algemene kinderpopulatie kon echter niet worden uitgesloten.

Tot slot zijn in **hoofdstuk 5** de belangrijkste resultaten en betekenis besproken van het onderzoek dat in dit proefschrift is beschreven. Ook valt in dit hoofdstuk te lezen welke vragen nog beantwoord dienen te worden om in de toekomst jonge kinderen wereldwijd zo goed mogelijk te kunnen beschermen tegen RSV-infectie. Met de bijdrage van dit proefschrift, de voortgang van verscheidene wetenschappelijke studies en de ontwikkeling van verschillende vaccinatiestrategieën, zal deze toekomst hopelijk nabij zijn.

7

Abbreviations
Contributing authors
Dankwoord
List of publications
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Abbreviations

ARR	Absolute risk reduction
ATS	American thoracic society
BCG	Bacillus Calmette-Guérin
CFR	Case fatality ratio
CHAMPS	Child health and mortality prevention surveillance
CI	Confidence interval
CrI	Credible interval
DTP	Diphtheria, tetanus, and pertussis
ERS	European respiratory society
FDA	US food and drug administration
FEF_{25-75}	Forced expiratory flow between 25% and 75% of vital capacity
FeNO	Fraction of exhaled nitric oxide
$FEV_{0.5}$	Forced expiratory volume in 0.5 s
FEV_1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
GA	Gestational age
GBD	Global burden of disease
GEN	Global epidemiology network
GOLD	Global online mortality database
Hib	Haemophilus influenzae type b
HIV	Human immunodeficiency virus
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IQR	Interquartile range
ISAAC	International study of asthma and allergies in childhood
LMP	Last menstrual period
LRTI	Lower respiratory tract infection
MMR	Measles, mumps, and rubella
NIH	National institutes of health
PCA	Palivizumab competing antibody
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PICU	Paediatric intensive care unit
RCT	Randomised controlled trial
Rint	Resistance of the respiratory system
RRR	Relative risk reduction

RSV	Respiratory syncytial virus
SAGE	Strategic advisory group of experts
SD	Standard deviation
TB	Tuberculosis
UI	Uncertainty interval
WBC	White blood count
WGA	Weeks' gestational age
WHO	World health organisation

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List of publications

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Curriculum vitae

Nienke Scheltema was born on the 3rd of September 1986 in Amsterdam, the Netherlands. In 2004 she graduated from secondary school (gymnasium) at the Baarnsch Lyceum. In the same year she started her medical training at the University of Utrecht. During medical school she developed her interest for global health during a clinical internship at the Saint Francis Hospital in Katete, Zambia, and she obtained a minor degree in Development Studies at the VU university in Amsterdam in 2010. This was followed by a research internship at the Amsterdam Institute of Global Health and Development under supervision of prof. dr. F.G.J. Cobelens. She graduated from medical school in 2011 and after travelling around South-America she started clinical work as a resident at the paediatric department of the Rijnstate Hospital in Arnhem. In 2013 she continued her clinical work in paediatrics at the Wilhelmina Children's Hospital in Utrecht, where she started her PhD research under supervision of prof. dr. L.J Bont and prof. dr. C.K van der Ent later that year. During her PhD she spent three months at the Usher Institute of Population Health Sciences and Informatics of Edinburgh University in Scotland, United Kingdom, where she was supervised by prof. H. Nair. The results of her PhD research projects have been described in this thesis. Nienke will commence her general practice training programme at the University Medical Center Utrecht in September 2018.