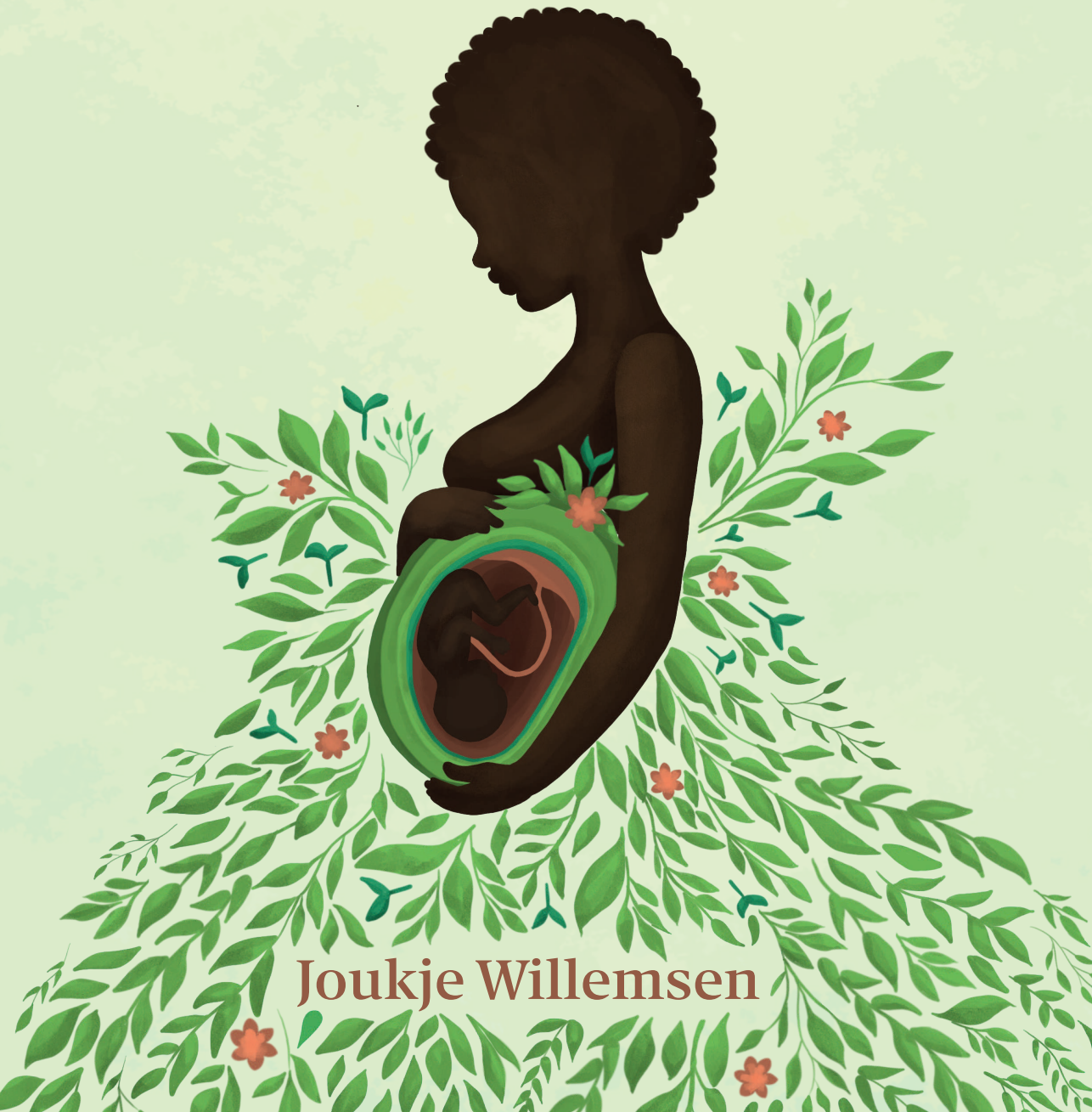
# Collaborating with *Mother Nature*

## The potential impact of maternal vaccination on RSV-related mortality



Joukje Willemsen

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**COLLABORATING WITH MOTHER NATURE**

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# Collaborating with Mother Nature

The potential impact of maternal vaccination on RSV-related mortality

**In samenwerking met Moeder Natuur**

De potentiële impact van maternale vaccinatie op RSV-gerelateerde sterfte

(met een samenvatting in het Nederlands)

**Proefschrift**

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# Chapter 1

## General Introduction



## General context

Respiratory syncytial virus (RSV) was first isolated in 1956 from material collected from a chimpanzee with a runny nose [1], and in 1961 it was identified for the first time in infants and children during a bronchiolitis epidemic [2]. RSV is an enveloped, single-stranded RNA virus that belongs to the Pneumoviradae family [3]. It circulates globally as two subtypes, RSV-A and RSV-B. RSV is transmitted primarily through close contact via inhalation of large respiratory droplets generated by infected individuals or through self-inoculation after contact with contaminated surfaces [3,4]. Although the majority of RSV infections are mild, some cases progress to severe lower respiratory tract infections (LRTIs), most commonly manifesting as bronchiolitis in infants. Bronchiolitis presents with cough, wheeze, increased work of breathing, poor feeding, and occasionally apnea [3,5]. Excessive mucus production can obstruct small airways leading to rapid respiratory deterioration or even death.

Natural immunity acquired after infection is incomplete and short-lived, which means reinfections are common throughout life [6]. RSV typically circulates in winter epidemics in temperate regions and during the rainy season in tropical climates [7]. Primary RSV infection typically occurs during an infant's first RSV season, and nearly all children have been infected by the age of two [8,9]. Disease severity is highest in infants and in older adults [6]. For infants, underlying risk factors for severe RSV disease include prematurity, neurological disorders, Down syndrome, chronic lung disease, immunodeficiency, and congenital heart disease [5,10]. However, most children hospitalized with RSV are born at term and have no underlying medical conditions; in this group, younger age is the most significant risk factor.

Treatment remains largely supportive; there is no approved therapy that reliably shortens the disease course or halts progression to life-threatening disease, and in severe cases ventilatory support and admission to pediatric intensive care may be required [5]. In the course of this PhD research (2020-2025), the field of RSV prevention witnessed major advancements with the market approval of multiple RSV immunizations and their implementation in several countries.

## Global burden and mortality

LRTIs are the leading cause of death among children under five years of age outside the neonatal period, accounting for approximately 12% of childhood deaths worldwide [4,11]. The majority of LRTIs in young children are viral in origin, with RSV responsible for the largest share (31%) [11]. The global incidence of RSV respiratory tract infection (RTI) is unevenly distributed, with the highest burden observed in low- and middle-income countries (LMICs) [5]. Infants younger than 6 months have the highest disease burden.

A global burden study from 2022 [12] presented updated yearly global burden estimates on RSV-related acute LRTI infections, hospital admissions and deaths (**Table 1**). Notably, the authors found that more than 95% of RSV-associated acute LRTI episodes and over 97% of RSV-attributable deaths across all age groups occurred in LMICs. A major challenge to accurate burden estimation is that many deaths occur outside health facilities, especially in LMICs, and thus are not captured by hospital-based surveillance systems [5].

**Table 1.** RSV-associated global burden estimates from Li et al. [12].

| Outcome | 0 - 60 months burden estimate | 0 - 6 months burden estimate |
|---|---|---|
| Acute LRTI infections (95% UR) | 33.0 million (25.4-44.6 million) | 6.6 million (4.6-9.7 million) |
| Hospital admissions (95% UR) | 3.6 million (2.9-4.6 million) | 1.4 million (1.0-2.0 million) |
| Hospital deaths (95% UR) | 26,300 (15,100 – 49,100) | 13,300 (6,800 – 28,100) |
| Overall deaths* (95% UR) | 101,400 (84,500 – 125,200) | 45,700 (38,400-55,900) |

UR: uncertainty range

*Including out-of-hospital deaths

## Prematurity as risk factor

Preterm infants (born before 37 weeks of pregnancy) are especially vulnerable to severe RSV infections because their immune systems are not fully developed, their airways are smaller, they receive fewer protective antibodies from their mothers, and they are more likely to have ongoing lung problems compared with babies born at full term[13]. Globally, preterm births account for more than one in ten livebirths, with regional estimates ranging from 9% to 13% [14]. Children born prematurely contribute disproportionately to RSV-related mortality: preterm infants account for an estimated 40% (95% uncertainty range [UR] 17–65) of RSV-attributable deaths in infancy, corresponding to approximately 26,760 (11,190–46,240) deaths worldwide in 2019 [13].

## Antibody transfer and early-life RSV protection

RSV protection in early life is driven largely by antibody-mediated immunity. Antibodies, or immunoglobulins, are produced by plasma cells and function by binding specific viral targets, enabling viral neutralization or clearance [15]. Among the different antibody classes, immunoglobulin G (IgG) is the most relevant for infant protection because it is the dominant circulating isotype and the only one that efficiently crosses the placenta. Although infants produce small amounts of IgG early in life, endogenous IgG production only starts to increase substantially from around 4–6 months of age (**Figure 1**). In a healthy pregnancy, IgG antibodies are actively transported across the placenta from the maternal to the fetal circulation, beginning in the early second trimester. As transfer becomes more efficient later in pregnancy, premature infants are born with lower antibody levels than term babies (**Figure 1B**). By the time of birth, antibody levels in full-term infants often exceed those of the mother, providing passive protection during a period of high vulnerability [16]. Transplacental antibody transfer occurs via a specific neonatal IgG receptor expressed on placental cells [15]. The efficiency of this transfer is influenced by factors such as the IgG subtype induced by vaccination, maternal hypergammaglobulinemia, and maternal infections, including HIV and malaria [16].

## Infant immunization strategies and prevention

Early RSV prevention efforts were shaped by the failure of a formalin-inactivated RSV vaccine tested in the 1960s, which led to enhanced respiratory disease in vaccine recipients after natural infection, including two fatalities [4,17,18]. This tragic outcome slowed RSV vaccine development for several decades. Vaccination in the neonatal period is challenging because neonates may generate suboptimal protective immune responses, and maternally derived antibodies can blunt the response to vaccination [19].

*Monoclonal antibodies*

Palivizumab, a monoclonal antibody, was the only licensed option for RSV-prevention from 1998 until 2023 [5]. Monoclonal antibodies are laboratory-produced antibodies given directly to the infant to provide immediate, short-term protection through passive immunization. Although palivizumab effectively reduces RSV-related hospitalizations, its use has largely been restricted to high-risk infants in high-income countries due to high costs and the need for monthly dosing during the RSV season. In early 2023, nirsevimab, a monoclonal antibody with an extended half-life, received market approval in Europe and the USA for all infants [5,17]. Clesrovimab, a second long-acting monoclonal antibody, was approved by the Food and Drug Administration (FDA) in June, 2025 [17]. However, their relatively high cost is likely to limit widespread access in some LMICs [17].

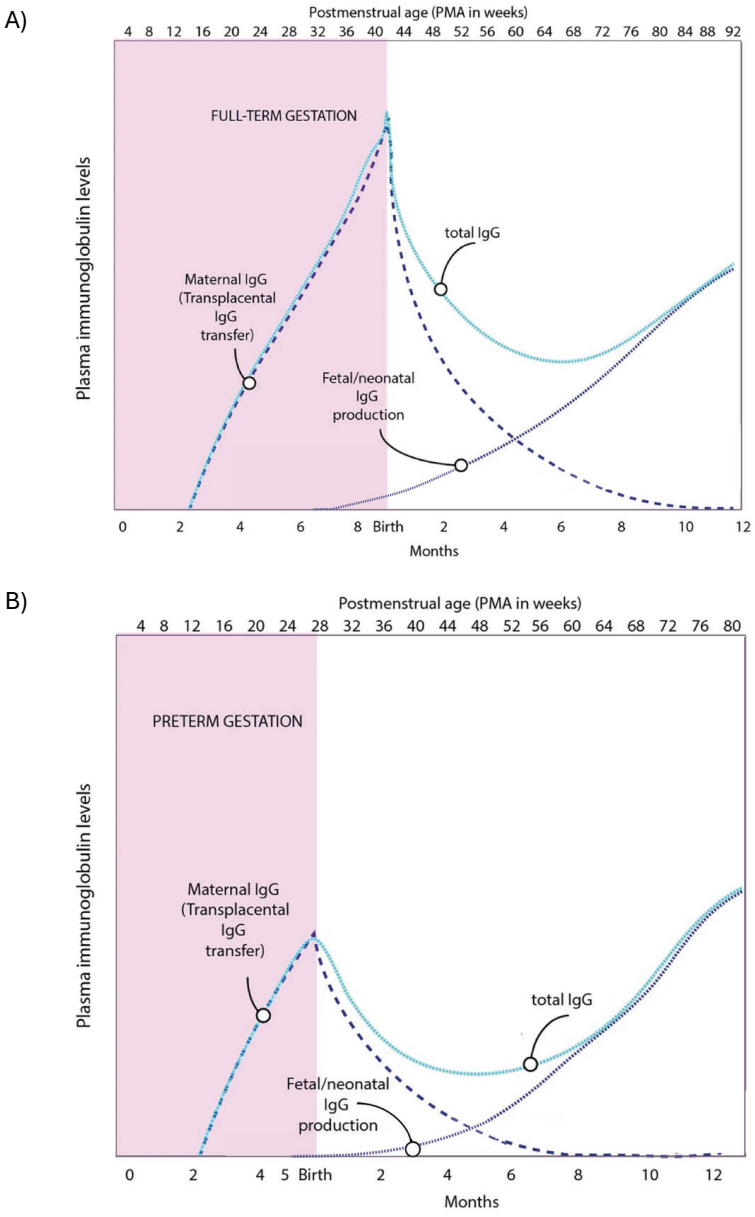



**Figure 1**: IgG concentration in a (A) full term and (B) preterm infant during pregnancy up until 1 year. Modified from Darmawan et al. [15]

*Maternal vaccination*

Vaccination during pregnancy is an alternative strategy to protect infants against RSV. Maternal vaccination has proven to be highly effective for preventing infection in early life and has been successfully implemented for tetanus, influenza, and pertussis [7,19]. The RSV prefusion F (RSVpreF) vaccine, administered during pregnancy to protect infants from severe RSV disease after birth, has been approved in several countries since 2023 [5].

Maternal vaccination stimulates the mother's immune system to generate RSV-specific antibodies and builds on the natural transplacental transfer of antibodies from mother to fetus during pregnancy [16]. Although this protection is temporary, maternal vaccination can protect infants from birth through the first months of life, a period of highest vulnerability, making it particularly relevant in LMICs, where the burden of RSV is greatest [7].

## Impact modelling

In LMICs, the public health burden is substantial and resources are limited. Thus, it is critical that governments and large funding organizations are informed about effectiveness of new interventions so that interventions that have the highest impact and best value for money can be prioritized [20]. Although vaccine efficacy is established in controlled clinical trials, trial data alone are often insufficient to extrapolate performance across diverse epidemiological and health system settings. In addition, the effectiveness of maternal vaccines in specific risk groups, such as preterm infants, is often poorly estimated due to limited statistical power in trials. Mathematical models provide a framework to evaluate the potential impact and cost-effectiveness of preventive strategies across settings and populations, informing policy decisions and funding priorities for governments and organisations such as Gavi, the Vaccine Alliance. This thesis builds on the mathematical model developed by Scheltema et al. [21], a model for maternal vaccine-induced antibody dynamics, taking into account transplacental antibody transfer rates and antibody decline after birth **(Box 1)**.

**Box 1. Simulation of fetal and neonatal antibody trajectories following maternal vaccination (Scheltema et al. [21])**

For each child with known gestational age and age at death, a counterfactual scenario is simulated in which the mother is assumed to have received vaccination during pregnancy, to assess whether maternal immunization could have prevented RSV-related mortality. The model incorporates gestational age–dependent transplacental antibody transfer and the postnatal decay of maternally derived antibodies (**Figure 2**). Following maternal vaccination, maternal antibody levels are assumed to increase, leading to a corresponding elevation in fetal antibody concentrations. After birth, neonatal antibody levels are modeled to decline over time as a result of antibody decay, until the time of observed death. An RSV-related death is considered prevented, or at least delayed, if the simulated vaccine-derived antibody level exceeds the protective threshold at the time of death. The authors report the proportion of deaths that could potentially be prevented under different assumptions regarding the timing of maternal vaccination across gestational weeks.

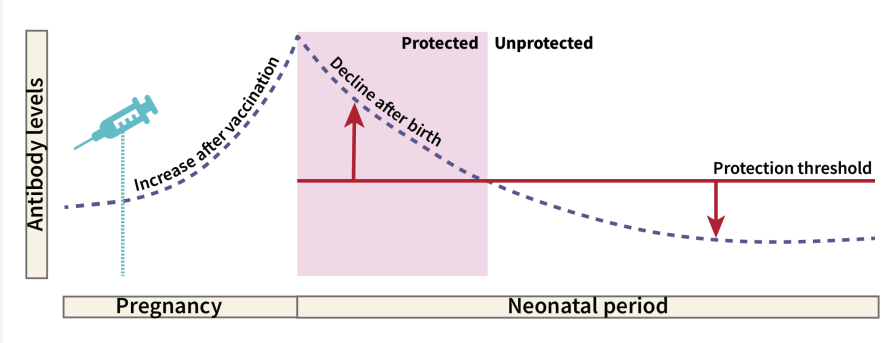



**Figure 2**: Example illustration of modeled individual antibody trajectories over time and their comparison with the protective threshold for protection.

## Gavi, the Vaccine Alliance

While new RSV vaccines are being introduced in high-income countries, many LMICs remain reliant on support from Gavi, the Vaccine Alliance, to access these interventions [18,22]. Gavi is an international organization dedicated to improving equitable access to new and underused vaccines in LMICs. Every five years, the Vaccine Investment Strategy guides Gavi's vaccine support decisions based on expected impact, cost, value for money, and programmatic feasibility in Gavi-eligible countries [22]. In its 2018 Vaccine Investment Strategy for the 2021–2025 funding period, Gavi evaluated several RSV prevention strategies under development, including maternal vaccination and monoclonal antibodies [23]. One of the key criteria used to prioritize these interventions was their projected health impact, measured by the number of cases and deaths averted. In July, 2025, Gavi announced its decision to support a maternal RSV vaccination program for eligible LMICs [24].

## Thesis objective and structure

The main objective of this thesis is to inform cost-effectiveness modelers and decision-makers about the potential impact of maternal RSV vaccination on RSV-related mortality in LMICs. To achieve this, we refined and extended an existing mathematical model (**Box 1**) and updated key inputs and assumptions.

**Part I** characterizes RSV-related age-at-death distributions across infancy, which are critical for estimating the proportion of mortality that can be averted. In **Chapter 1** we characterize and compare the age-distribution of both in-hospital and community deaths from LMICs. In **Chapter 2** we estimate the age-distribution of RSV deaths in Gavi-eligible countries, an essential input for vaccine-impact models.

**Part II** describes the updated mathematical impact model and its predicted impact. **Chapter 3** models the potential impact of maternal vaccination in Gavi-eligible countries. **Chapter 4** examines differences in regulatory decision-making between the FDA and the European Medicines Agency (EMA).

**Part III** refines the immunological evidence base by characterizing antibody decay in early life, and the relation between placental health and maternal antibody transfer. **Chapter 5** challenges the assumption that preterm and full-term infants have similar maternal antibody half-lives. **Chapter 6** investigates whether reduced maternal antibody transfer might be partially explained by placental abnormalities.

**Part IV** explores how innovations in trial design and multi-endpoint analysis support regulatory and post-licensure decision-making. **Chapter 7** summarizes a meeting report on optimizing clinical trial design. **Chapter 8** introduces a novel framework for multi-endpoint efficacy evaluation.

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# Part I

## Age-at-death distribution



"Moral ambition is the desire to really make a difference.
It's not a character trade. It's a mindset; an infectious mindset"

- Rutger Bregman

# Chapter 2

## Global Respiratory Syncytial Virus–Related Infant Community Deaths

Natalie I Mazur, Yvette N Löwensteyn, Joukje E Willemsen, Christopher J Gill, Leah Forman, Lawrence M Mwananyanda, Dianna M Blau, Robert F Breiman, Shabir A Madhi, Sana Mahtab, Emily S Gurley, Shams El Arifeen, Nega Assefa, J Anthony G Scott, Dickens Onyango, Beth A Tippet Barr, Karen L Kotloff, Samba O Sow, Inacio Mandomando, Ikechukwu Ogbuanu, Amara Jambai, Quique Bassat, CHAMPS Network the RSV GOLD Study Group, Mauricio T Caballero, Fernando P Polack, Saad Omer, Abdul Momin Kazi, Eric A F Simões, Ashish Satav, Louis J Bont

Clin Infect Dis. 2021 Sep 2;73(Suppl_3):S229-S237

## ABSTRACT

**Introduction**:

Respiratory syncytial virus (RSV) is a leading cause of pediatric death, with >99% of mortality occurring in low- and lower-middle income countries. At least half of RSV-related deaths are estimated to occur in the community, but clinical characteristics of this group of children remain poorly characterized.

**Methods**:

The RSV Global Online Mortality Database (RSV GOLD), a global registry of under-5 children who have died with RSV-related illness, describes clinical characteristics of children dying of RSV through global data sharing. RSV GOLD acts as a collaborative platform for global deaths, including community mortality studies described in this supplement. We aimed to compare the age distribution of infant deaths <6 months occurring in the community with in-hospital.

**Results**:

We studied 829 RSV-related deaths <1 year of age from 38 developing countries, including 166 community deaths from 12 countries. There were 629 deaths that occurred <6 months, of which 156 (25%) occurred in the community. Among infants who died before 6 months of age, median age at death in the community (1.5 months; IQR: 0.8-3.3) was lower than in-hospital (2.4 months; IQR: 1.5-4.0; $p<0.0001$). The proportion of neonatal deaths was higher in the community (29%, 46/156) than in-hospital (12%, 57/473, $p<0.0001$).

**Conclusions**:

We observed that children in the community die at a younger age. We expect that maternal vaccination or immunoprophylaxis against RSV will have a larger impact on RSV-related mortality in the community than in-hospital. This case series of RSV-related community deaths, made possible through global data sharing, allowed us to assess the potential impact of future RSV vaccines.

## INTRODUCTION

As part of the global agenda for 2030 set by the United Nations, Sustainable Development Goal (SDG) 3 urgently calls for ending preventable deaths of children under 5 years of age. Globally, respiratory syncytial virus (RSV) is a leading cause of death after malaria for infants.[1] More than 99% of these RSV pediatric deaths occur in the developing world.[2] Current global mortality estimates are almost exclusively based on in-hospital RSV mortality. However, it is likely that a significant proportion of these deaths occur outside the hospital, especially in low-income settings.[3] A recent meta-analysis estimated that out-of-hospital mortality was 2-fold higher than in-hospital mortality in 3 low-income and lower-middle-income countries (L(M)ICs).[3] Thus, the burden of out-of-hospital RSV deaths appears to be at least as high as in-hospital deaths. Despite the magnitude of the problem, understanding the clinical characteristics of pediatric RSV-related mortality in the community remains a key knowledge gap.

Addressing the knowledge gap on community deaths can give key insights to inform policy for a future RSV vaccine. More than 50 vaccine candidates are in clinical development for RSV.[4] Different approaches to RSV prevention confer varying degrees and duration of protection. Currently, 2 major approaches are in development for infants: (1) maternal vaccination and (2) passive antibody prophylaxis. Recent late-phase trial data show the potential degree and duration of protection for these different approaches, with infant monoclonals giving a higher degree and duration of protection than a maternal vaccine. The recently published phase III results of a post-fusion F protein maternal RSV vaccine show an antibody half-life of 49.1 days with 44.4% efficacy (95% confidence interval [CI]: 19.6-61.5%) against severe RSV lower respiratory tract infection (LRTI) through the first 3 months of life.[5] Prophylaxis with an extended half-life monoclonal antibody shows a longer duration of protection with 70.1% efficacy (95% CI: 52.3-81.2%) against RSV LRTI through the first 5 months of life.[6] To estimate the potential impact of RSV-preventive interventions against mortality in the developing world, it is essential to characterize children dying of RSV in the community.

Studying community deaths is difficult given the challenges associated with virological studies in deaths that occur in the community. To date, the largest case series of community RSV-related deaths includes 11 deaths at home in a single, urban setting in Argentina.[7] Although in-hospital deaths are challenging to capture in L(M)ICs given the lack of diagnostic capacity, capturing community deaths is even more challenging due to difficulty in ascertaining cause-of-death based on the low specificity of verbal autopsy data and difficulty obtaining postmortem patient samples. However, in the past years, several studies supported by the Bill & Melinda Gates Foundation (BMGF) aimed to measure RSV mortality in the community: Z-PRIME

(the Zambia Pertussis RSV Infant Mortality Estimation study); community-based studies in Argentina, India, and Pakistan; as well as the Child Health and Mortality Prevention Surveillance (CHAMPS) in South Africa, Bangladesh, Kenya, Mali, Mozambique, Ethiopia, and Sierra Leone.[8] The RSV Global Online Mortality Database (RSV GOLD) provides the unique opportunity to pool data from all of these studies and compare global individual-level patient data of children dying in the community to children dying in-hospital.

espiratory syncytial virus-preventive interventions aim to prevent infant death in accordance with the SDGs. Estimated impact of a maternal vaccine or infant monoclonal on pediatric deaths will guide policy decisions and accelerate access to life-saving interventions. The primary aim of this article is to describe global community pediatric deaths under 6 months and compare this group with in-hospital deaths in upper-middle-income countries (UMICs) and L(M)ICs.

## METHODS

*Study site, design, and population*

RSV GOLD is a global online registry for children under the age of 5 years who died with laboratory-confirmed RSV infection after 1 January 1995.[9] Individual patient-level data are collected using an online questionnaire. Variables collected in the RSV GOLD database have been published previously.[9] Data are collected through active outreach to researchers and physicians worldwide. Investigators of BMGF-funded community mortality studies were specifically asked to share data collected through 2 March 2021. The data from these community studies have been published in this supplement issue. Two community studies (Z-PRIME and Pakistan Community Mortality studies) included children younger than 6 months of age; other studies recruited children through at least 12 months of age (Supplemental Table 1). Data from studies submitted to the RSV GOLD registry were collected both prospectively and retrospectively.

In this analysis RSV-related deaths above 1 year of age, nosocomial deaths, and deaths in high-income countries were excluded (**Figure 1**). Based on the expected duration of protection for infant RSV-preventive interventions, the primary aim of this study was to compare the age distribution of RSV-related infant deaths under 6 months occurring in the community with those in-hospital. The secondary aim was to describe age at death for children dying of RSV in the first year of life in the community. In order to achieve our secondary aim, to describe the age distribution under 1 year, we analyze the population ("12m cohort"), in which we excluded 2 community studies that only enrolled children up to 6 months of age.

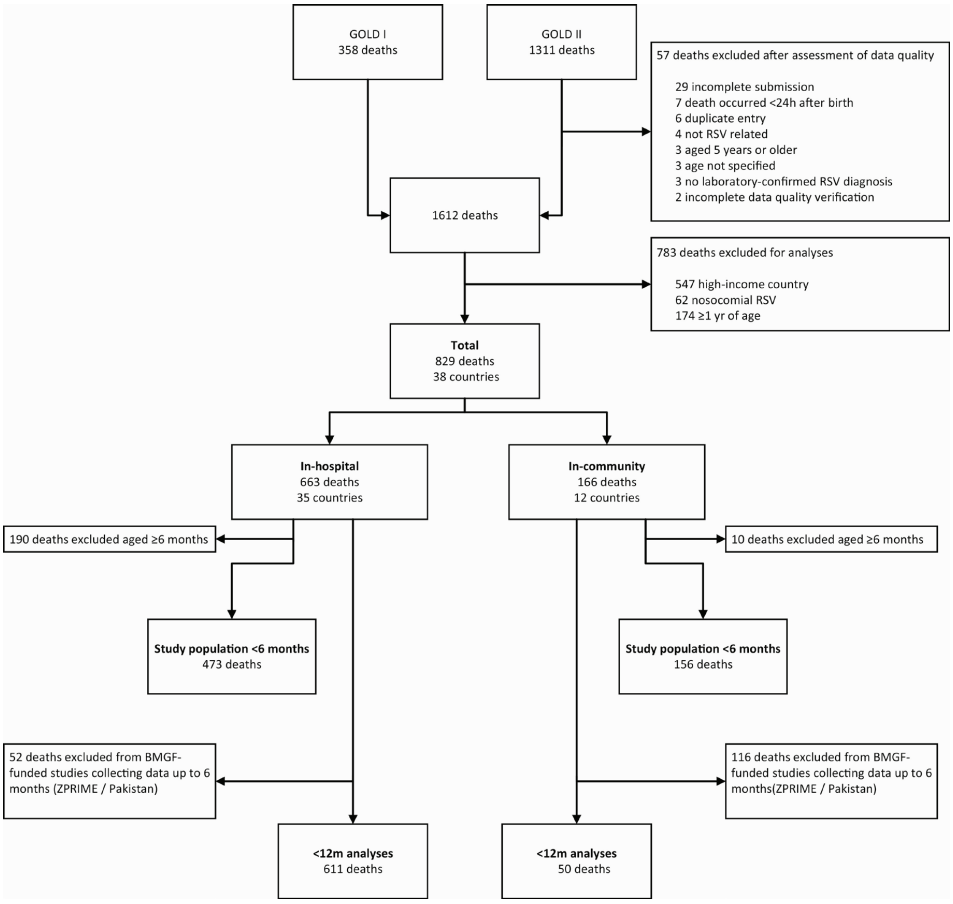



**Figure 1**. Flowchart of children included in this study. Flowchart shows children excluded via both data quality and per definition of study population. For the primary analysis we analyzed 629 children dying under age 6 months (473 in-hospital deaths and 156 community deaths). For the secondary analysis we analyzed 661 children dying under age 12 months (611 in-hospital deaths and 50 community deaths). GOLD I: Pediatric deaths published as a retrospective case series from 1 November 2014 to 31 October 2015.9 GOLD II includes pediatric deaths collected after this publication.

Abbreviations: m, months; BMGF, Bill & Melinda Gates Foundation; GOLD, Global Online Mortality Database; RSV, respiratory syncytial virus; ZPRIME, Zambia Pertussis RSV Infant Mortality Estimation Study

*Data collection and case definition*

Case definitions of a community death varied between different BMGF-funded community mortality studies (Supplemental Table 1). For community deaths submitted to RSV GOLD that did not originate from these studies, a community death was defined as a child who did not die in the hospital or a child who was not hospitalized and location of death was unknown (n = 2). As in our previous publications, we included any death with laboratory-confirmed RSV infection and did not require RSV to be the primary cause of death (Supplemental Table 5).[9] Neonates were defined as children through 1 month of age.

Upon submission to the database, data-quality checks were performed by the RSV GOLD team to ensure the completeness and accuracy of the data. To this end, case data were verified for missingness, plausibility, and accuracy through direct communication with collaborators as soon as possible after case submission. Minimum essential data for inclusion were the key variables age at death, year of death, and laboratory-confirmed RSV infection.

*Statistical analysis*

For continuous variables, the means or medians were reported and differences between 2 groups were tested with a Mann-Whitney U test. Categorical variables were described with frequencies and percentages and compared between groups using Fisher's exact test. We did not perform imputation for missing data because data were not missing for essential variables and for other variables there was no clear correlation on which to build a multiple imputation model.

We considered $p<0.05$ to be significant for all analyses. Despite multiple comparisons, we chose not to correct for an increased false-positive rate due to the exploratory nature of the study and small sample size. The statistical analysis was performed using R version 4.0.2 (R Core Team 2020, Vienna, Austria) with the following packages: ggplot2[10], ggpubr[11], rnaturalearthdata[12], dplyr[13] and qwraps2[14].

We performed 2 sensitivity analyses: (1) without the Z-PRIME data and (2) restricting the data to community mortality studies. As the majority of the data for community deaths originated from the Z-PRIME study in Zambia, we performed a sensitivity analysis that excluded the Z-PRIME cases to ensure that this overrepresentation of Zambia deaths did not lead to different results. Furthermore, we tested the assumption that community deaths from the Z-PRIME data are representative for community deaths from other L(M)ICs by testing the observed characteristics for significant differences.

*Ethical considerations*

The institutional research board of the University Medical Center Utrecht waived the requirement for parental informed consent in 2014 since the study concerns only anonymized secondary data. Collaborators sharing data were encouraged to adhere to local standards for ethics approval in accordance with the RSV GOLD Ethics Guideline.[15]

## RESULTS

*Study population*

The overall study population included 829 pediatric deaths under 1 year of age from 38 countries classified as UMIC or L(M)IC according to the World Bank income group classification (**Figure 1**, Supplemental Table 2). Of these, 166 deaths occurred in the community. The world maps in **Figure 2A** and **2B** show the global distribution of community and in-hospital deaths, respectively. The study population of infants under 6 months consisted of 629 deaths, of which 156 (25%) deaths from 12 different countries occurred in the community (Supplemental Table 4). Most community deaths were from Zambia (72%, 112/156). Community deaths were submitted from 2009 onwards, while data for in-hospital deaths were shared from 1995 onwards. The 12m cohort comprises 661 children, of whom 8% (50/661) died in the community and 92% (611/661) died in-hospital.

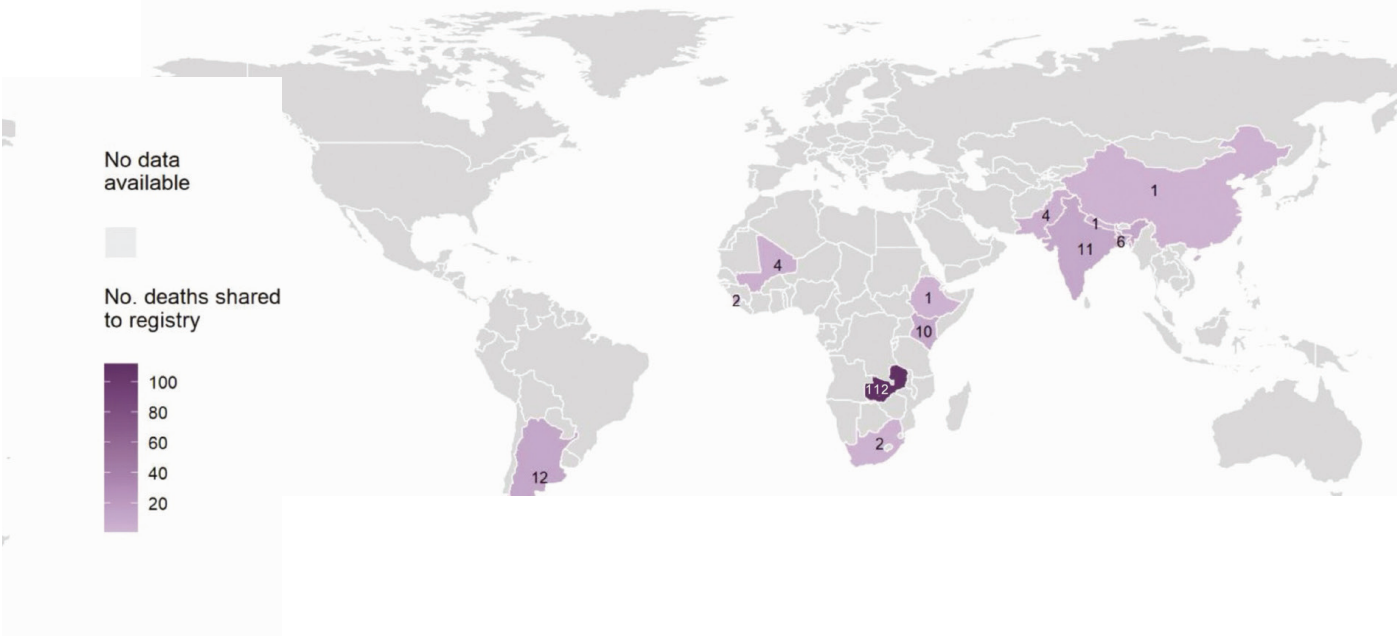



**Figure 2A**, World map showing L(M)ICs and UMICs that shared RSV-confirmed community deaths under 12 months of age and number of RSV-confirmed community deaths shared to the registry. The color gradient of purple indicates number of deaths shared, with darker purple representing increased number of deaths shared. Numbers of deaths are visible on the map.

*Abbreviations: L(M)ICs, lower-income-lower-middle-income country; RSV, respiratory syncytial virus; UMIC, upper-middle-income country.*

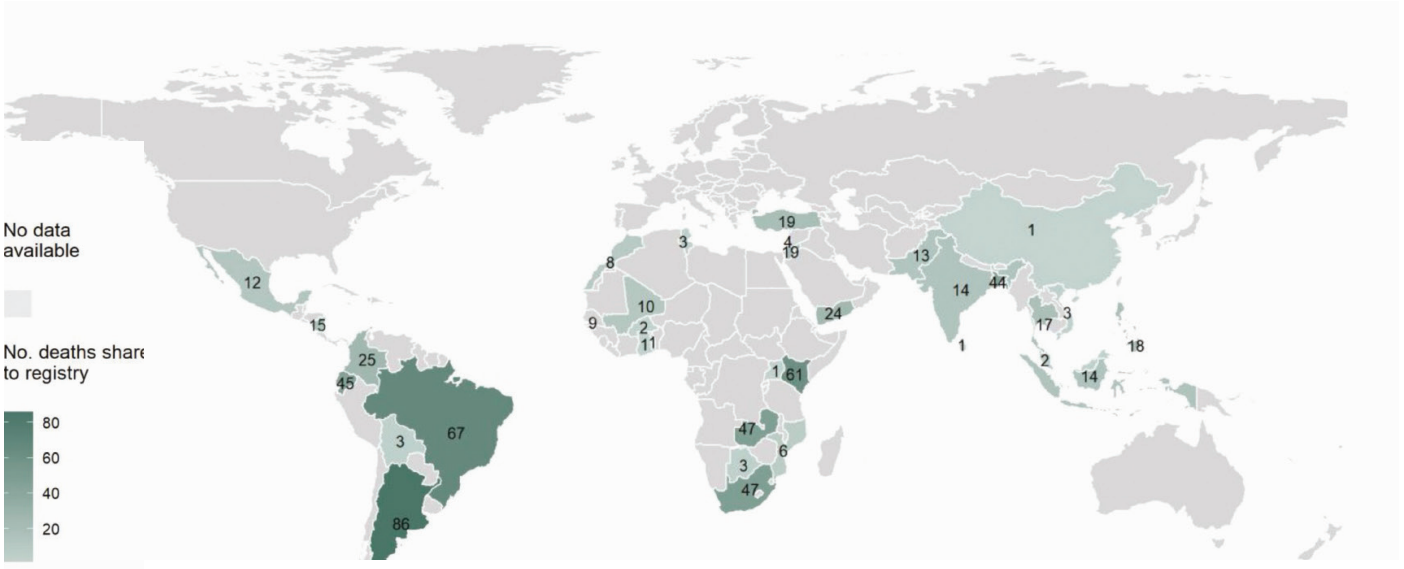



**Figure 2B**, World map showing L(M)ICs and UMICs that shared RSV-confirmed in-hospital deaths under 12 months of age and number of deaths of RSV-confirmed in- hospital deaths shared to the registry. The color gradient of green indicates number of deaths shared, with darker green representing increased number of deaths shared. Numbers of deaths are visible on the map.

*Abbreviations: L(M)ICs, lower-income-lower-middle-income country; RSV, respiratory syncytial virus; UMIC, upper-middle-income country.*

*Age at death of community versus in-hospital deaths*

Median age at death was significantly lower for community deaths (1.5 months; IQR: 0.8–3.3) than in-hospital deaths (2.4 months; IQR: 1.5–4.0; p<0.0001) (**Table 1**, **Figure 3A**). Deaths in the community included a higher proportion of neonates (29%, 46/156) than deaths occurring in-hospital (12%, 57/473; p<0.0001). Similar results were found for the 12m cohort (**Table 2**, **Figure 3A**). In the 12m cohort, median age at death was lower in the community (2.1 months; IQR: 1.3-5.0) compared with in-hospital (4.0 months; IQR: 2.0-6.1; p=0.02) (**Table 2**, **Figure 3B**). Similarly, for the 12m cohort, a higher proportion of deaths occurred in the neonatal period in the community (14%, 7/50) than in-hospital (7%, 40/611), although this difference was not statistically significant (p=0.08).

**Table 1.** Clinical characteristics for children <6 months who died with RSV in-hospital vs in-community in LMICs and UMICs

| Clinical characteristics | All deaths (N = 629) | Community (N = 156) | In-hospital (N = 473) | p-value |
|---|---|---|---|---|
| Sex, male; % (n/N) | 54 (330/615) | 55 (78/142) | 53 (252/473) | NS |
| Age at deaths, months; median (IQR) | 2.0 (1.1 - 4.0) | 1.5 (0.8 - 3.3) | 2.4 (1.5 - 4.0) | <0.0001 |
| Neonatal deaths; % (n/N) | 16 (103/629) | 29 (46/156) | 12 (57/473) | <0.0001 |
| Comorbidity; % (n/N) | 43 (173/403 ) | 31 (11/35) | 44 (162/368) | NS |
| Prematurity; % (n/N) | 31 (92/297) | 25 (11/44) | 32 (81/253) | NS |
| Gestational age, weeks; mean (SD, N) | 36.6 (3.5, 145) | 38.5 (2.4, 23) | 36.2 (3.6, 122) | 0.005 |
| Birth weight, kilograms; median (IQR, N) | 2.8 (2.2 - 3.2,156) | 3.0 (2.4 - 3.3, 30) | 2.8 (2.2 - 3.2, 126) | NS |
| Month & year of death; min/max | Jul 1995 - Feb 2021 | Feb 2009 - Jul 2020 | Jul 1995 - Feb 2021 | - |
| Not immunized; % (n/N) | 30 (71/235) | 36 (15/42) | 29 (56/193) | NS |
| Other children in household; % (n/N) | 75 (118/158) | 82 (18/22) | 74 (100/136) | NS |
| Mother uneducated; % (n/N) | 10 (23/231) | 6 (6/103) | 13 (17/128) | NS |
| Father uneducated; % (n/N) | 6 (9/161) | 1 (1/86) | 11 (8/75) | 0.01 |

P-value is provided for the comparison between community and in-hospital deaths.

*Abbreviations: LMICs lower-middle income countries, UMICs upper-middle income countries, RSV respiratory syncytial virus, NS not significant.*

**Table 2.** Clinical characteristics for children <12 months who died with RSV in-hospital vs in-community in LMICs and UMICs, excluding deaths from studies recruiting only <6m

| Clinical Characteristics | All deaths (N = 661) | Community (N = 50) | In-hospital (N = 611) | p-value |
|---|---|---|---|---|
| Sex, male; % (n/N) | 56 (369/661) | 56 (28/50) | 56 (341/611) | NS |
| Age at death, months; median (IQR) | 4.0 (2.0 - 6.0) | 2.1 (1.3 - 5.0) | 4.0 (2.0 - 6.1) | 0.02 |
| Neonatal deaths; % (n/N) | 7 (47/661) | 14 (7/50) | 7 (40/611) | NS |
| Deaths <6m; % (n/N) | 70 (461/661) | 80 (40/50) | 69 (421/611) | NS |
| Comorbidity; % (n/N) | 45 (250/561) | 28 (10/36) | 46 (240/525) | 0.04 |
| Prematurity; % (n/N) | 28 (101/356) | 24 (9/37) | 29 (92/319) | NS |
| Gestational age, weeks; mean (SD, N) | 36.6 (3.5, 195) | 38.4 (2.5, 27) | 36.3 (3.5, 168) | 0.01 |
| Birth weight, kilograms; median (IQR, N) | 2.8 (2.2 - 3.2, 208) | 3.0 (2.5 - 3.3, 30) | 2.8 (2.2 - 3.2, 178) | NS |
| Month & year of death; min/max | Jul 1995 – Feb 2021 | Feb 2009 - Feb 2020 | Jul 1995 – Feb 2021 | - |
| Not immunized; % (n/N) | 13 (33/258) | 19 (5/27) | 12 (28/231) | NS |
| Other children in household; % (n/N) | 73 (160/220) | 90 (19/21) | 71 (141/199) | NS |
| Mother uneducated; % (n/N) | 12 (19/155) | 8 (2/25) | 13 (17/130) | NS |
| Father uneducated; % (n/N) | 7 (6/81) | 5 (1/21) | 8 (5/60) | NS |

P-value is provided for the comparison between community and in-hospital deaths.

*Abbreviations: LMICs lower-middle income countries, UMICs upper-middle income countries, RSV respiratory syncytial virus, NS not significant.*

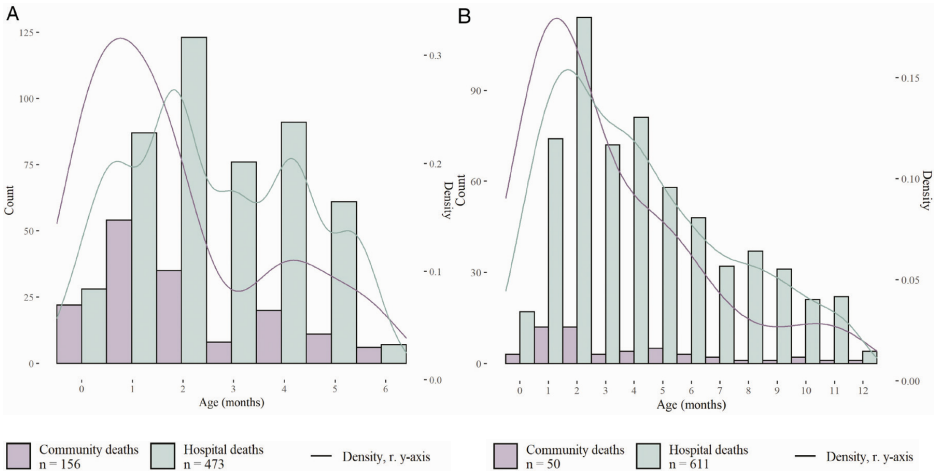



**Figure 3**. **A**, Histogram and density plot of age at death for children under 6 months who died with RSV in the community compared with in-hospital in L(M)ICs and UMICs. The histogram shows number of deaths (count, left y-axis) shared to the registry by age at death in months (rounded to the nearest integer) from age 0 up to 6 months for all infants under 6 months of age. Lines show the kernel density estimate of age at death in months (density, right y-axis). Deaths that occurred in the community are shown in purple, while deaths that occurred in the hospital are shown in green. **B**, Histogram and density plot of age at RSV-related death for children under 12 months who died in the community compared with in-hospital in L(M)ICs and UMICs. The histogram shows number of deaths shared (count, left y-axis) to the registry by age at death from age 0 up to 12 months for the 12m cohort. Lines show the kernel density estimate of age at death in months (density, right y-axis). Deaths that occurred in the community are shown in purple, while deaths that occurred in the hospital are shown in green.

*Abbreviations: L(M)ICs, lower income and lower middle income country; RSV, respiratory syncytial virus; UMIC, upper-middle-income country.*

*Clinical characteristics of community vs in-hospital deaths*

For infants who died under 6 months, clinical characteristics of community and in-hospital deaths were largely comparable. For children under 6 months with comorbidity data, 31% (11/35) of infants dying in the community had a comorbidity compared with 44% (162/368) who died in-hospital. However, data on comorbidities were missing for 78% of community deaths, limiting the power to analyze this characteristic (comorbidities are specified in Supplemental Table 8). The proportion of premature infants did not differ significantly between community (25%, 11/44) and in-hospital (32%, 81/253; not significant) deaths. We note that prematurity data were missing for a substantial proportion of community (72% 112/156) and in-hospital (47%, 220/473) deaths for infants under 6 months. The reported mean gestational age was lower for deaths in-hospital compared with those occurring in the community (36.2 vs 38.5 weeks; p=0.005).

The secondary analysis of the 12m cohort was remarkably similar to the primary analysis of children dying before age 6 months. Among infants dying in the community, 28% (10/36) had a comorbidity compared with 46% (240/525, p=0.04) of infants dying in-hospital. In the 12m cohort, the proportion of premature infants did not differ significantly between community versus in-hospital deaths (**Table 2**), although reported gestational age was significantly lower for infants dying in-hospital than in the community (36.3 vs 38.4 weeks; p=0.006).

*Sensitivity analyses*

We performed a sensitivity analysis excluding the majority of the community deaths, which originated from a single Zambian study site (71%, 112/156). The age at death in the community did not differ significantly for children who died in Zambia (n = 112; data shown in Gill et al in this supplement issue) compared with children from other countries (n = 44; 2.0 months; IQR: 1.3-3.3 months). The proportion of children with prematurity was similar for community deaths in Zambia and community deaths elsewhere (Forman et al, data published elsewhere in this supplement issue). After excluding the Zambia data, we found that age at death remained lower for children who died in the community (2.0 months; IQR: 1.3-3.3) compared with children who died in-hospital (2.5 months; IQR: 1.8-4.0), although this difference was not statistically significant (p=0.07) (Supplemental Table 6). The proportion of neonates was similar in the in-hospital and community deaths (Supplemental Table 6).

We performed a sensitivity analysis restricted to data obtained from the community mortality study sites (144 community deaths and 68 in-hospital deaths) to rule out bias due to differences in methodology of data collection, because data in this subset were collected systematically in the community and in the hospital setting

(Supplemental Table 7). In this analysis, we observed a lower median age at death in the community compared with in-hospital, although differences were smaller than in the main analysis and not statistically significant (1.5 vs 2.0 months; p=0.26). Moreover, in this sensitivity analysis, the proportion of neonates was similar in the in-hospital and community deaths (Supplemental Table 7).

## DISCUSSION

As a result of global data sharing by collaborators, this study is the first global case series to compare RSV-related mortality in the community with in-hospital deaths in L(M) ICs and UMICs. The aim of this study was to understand differences between infants dying in the community and infants dying in-hospital in order to inform RSV vaccine-development strategies for low-resource settings. We found that children dying in the community were generally younger than children dying in-hospital. A larger proportion of deaths in the community involved neonates in the primary analyses but not the sensitivity analyses, possibly due to a larger proportion of deaths originating from L(M) ICs in the community. The younger age at death in the community may be explained by difficulty of caregivers in recognizing respiratory danger signs at a younger age, resulting in delayed or no access to care for younger children with RSV LRTI. We conclude that RSV-prevention strategies targeting infants in the first months of life will likely have a larger impact on mortality occurring in the community than in-hospital. Thus, we expect a high impact of infant RSV immunization strategies via maternal vaccination or infant immunoprophylaxis.

The RSV GOLD database serves as a platform that can bundle data from study sites around the world to allow for a high-level analysis of RSV-related mortality around the globe. Previous publications on community deaths did not describe age distribution for RSV-related illness but instead described risk factors for community deaths in Argentina[7], leading causes of deaths determined by minimally invasive autopsies in the CHAMPS sites[8], and estimates of the proportion of out-of-hospital deaths in South Africa.[16] Previously we published a case series of in-hospital deaths in which we found the median age at death to be 5 months in L(M)ICs and 4 months in UMICs in children under 5 years of age. In this analysis, we found the median age at RSV-related death in infants from UMICs and L(M)ICs who died before 1 year of age to be similar (4.0 months; IQR: 2.0–6.1).

There were several limitations of this study. First, the community mortality studies contributing to the RSV GOLD registry were not designed identically and used different definitions for community deaths (Supplemental Table 1). A second limitation concerns the reporting of age at death by collaborators. There are 2 ways in which collaborators may have rounded age at death to age in months, which could introduce bias in our analysis. Due to general conceptualization of age, collaborators may have rounded age in months down. This rounding method may have introduced systematic

bias for the group of children who died in-hospital because age at death was most frequently shared in months for in-hospital deaths and in days for community deaths. Second, collaborators may round age to the nearest integer, which would mean that the cutoffs applied for our analyses exclude children whose age was rounded to 1 (neonates), 6 (primary analysis), or 12 (secondary analysis) months. For example, the observed difference in proportion of neonatal deaths could be influenced by misclassification bias. To this end, age reported in months may have been rounded to age 1 month for deaths in the first month of life and subsequently these children would not be classified as neonatal deaths more often for the in-hospital group. In summary, for both rounding methods, age at death may have been underestimated for children dying in the hospital, which would mean that the difference in age between community and in-hospital deaths may have been underestimated. A third limitation of the study is the quality and completeness of the data. An inherent weakness in global data sharing is that primary data cannot be verified and data-collection systems differ in quality. With extensive data-quality checks and direct verification with collaborators, we attempted to limit the impact of this methodological weakness. For some variables (comorbidity, prematurity), a high proportion of data were missing.

More than 50% of community death data originate from a single study site (Zambia). Our conclusions regarding age at death did not change when excluding these deaths from the analysis. An important limitation is the difficulty of measuring mortality in the community, which may have resulted in missed deaths. Data from the community were from a small number of countries while hospital deaths were shared from a larger number of countries over a longer period of time, which may account for differences in the data. Data from most studies were obtained from systematic postmortem sampling, which may not be comparable to the way in which data were obtained for the in-hospital group and which could explain the different findings in the sensitivity analyses. For this reason, the age difference could also be explained by limitations in study methodology as children with RSV may present with nonspecific symptoms to the hospital at a younger age and not be tested in L(M)ICs. However, in a sensitivity analyses limited to comparable groups in-hospital and in the community, we observed the same trend of lower age at death in the community. Despite data-quality verification processes, there are major limitations of the study methodology as published previously.[9]

Future steps should consist of analysis of a larger case series including more community deaths and a larger global distribution, which will allow for more robust conclusions regarding vaccine impact on infant mortality. Prospective, real-time data sharing of RSV-related death in L(M)ICs will contribute to increased data quality and completeness of data, including more detailed information on age at death, allowing for a better comparison between community and in-hospital RSV-related deaths. A uniform definition of RSV-related deaths in the community will allow for better collection and understanding of global community mortality. Future studies would be strengthened by enhanced systems for data collection of key clinical characteristics

such as immunization status, prematurity, and comorbidity for community deaths, as this information was frequently missing for this population.

## CONCLUSIONS

Community deaths are thought to represent more than half of all RSV-related deaths globally.[17] Characterizing these deaths is essential to estimate the impact of future preventive interventions. Due to global data sharing and efforts of BMGF-funded community mortality studies and other collaborators, the RSV GOLD database has served as a platform to aggregate robust data for analysis of RSV-related pediatric mortality on a global level. We show that infants under 6 months of age die at a younger age in the community than in-hospital. Modeling studies will have to translate these findings into expected impact of upcoming maternal vaccines and next-generation monoclonal antibodies against RSV.

For the first time, we show evidence that maternal vaccination or infant monoclonal prophylaxis may have a greater impact on RSV-related community mortality than in-hospital mortality. Ultimately, clinical trials and post-marketing surveillance studies will provide further evidence to evaluate the impact of these interventions on pediatric RSV mortality in the community versus in-hospital.

## NOTES

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*Author contributions*

LJB, NIM, and YNL conceptualized the study. NIM prepared the first version of the manuscript. YNL performed all data cleaning and preparation and assisted NIM with drafting the initial manuscript. JEW conducted all data analyses, created the figures, and assisted with interpretation of the results. LJB assisted with data collection. CJG, LF, LMM, DMB, RFB, SAM, SM, ESG, SEA, NA, JAGS, DO, BATB, KLK, SOS, IM, IO, AJ, QB, CHAMPS Network the RSV GOLD Study Group, MTC, FPP, SO, AMK, EAFS, and AS contributed data and provided critical feedback on the manuscript. All authors reviewed and approved the final version of the manuscript.

*Disclaimer*

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention, USA or the National Institute for Communicable Diseases, South Africa or the World Health Organization, or reflect positions or policies of the Bill & Melinda Gates Foundation.

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## SUPPLEMENTARY MATERIALS



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# Chapter 3

# Characteristics of inpatient and outpatient respiratory syncytial virus mortality in Gavi-eligible countries

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## ABSTRACT

**Introduction**:

The current understanding of the RSV-related mortality age distribution in low- and lower-middle-income countries (LMICs) relies on a limited number of disease incidence studies reporting wide age bands, and lacking specificity to Gavi-eligible countries. Understanding the age distribution of RSV-related deaths is crucial for the implementation of RSV interventions in LMICs that rely on support from Gavi. This study aims to provide the age profile of RSV mortality specifically in Gavi-eligible countries.

**Methods**:

Utilizing data from the RSV GOLD project, an ongoing global online mortality registry focusing on children under the age of 5 with laboratory-confirmed RSV infection, we employed two models (Complete Data Model and Prospective Data Model) to estimate the age profiles. To mitigate biases related to age group representation, we applied post-stratification weighting in our analysis.

**Results**:

We included 423 pediatric deaths, including 145 from the community, under 2 years of age from 15 Gavi-eligible countries. Both models identified a peak age at 1 month and found that the majority of RSV-related mortality cases (59–77 %) from Gavi-eligible countries occur before 6 months of life. However, the models exhibited disparities in other age-related metrics. We present fitted age-at-time-of-death probability distributions to aid impact and cost-effectiveness studies.

**Conclusions**:

We expect that implementing infant RSV immunization strategies, such as maternal vaccination or infant immunoprophylaxis, will have high impact on RSV-related mortality in Gavi-eligible countries. The divergent results from the two models underscore the importance of carefully considering potential biases in retrospective and surveillance data when interpreting the age profile of RSV mortality cases in future research.

## INTRODUCTION

Respiratory syncytial virus (RSV) infection is a leading cause of hospitalization and mortality worldwide in children under 5 years of age due to lower respiratory tract infection (LRTI)[1]. The majority of deaths (97%) occur in low- and lower-middle-income countries (LMICs) due to the poor accessibility and affordability of healthcare and poor quality of care in health facilities. Gaining insight into the age distribution of children who experience fatal outcomes due to RSV has become more pressing with ongoing research into prevention methods, such as vaccines and monoclonal antibodies, and policy debates on prophylaxis in resource-limited settings[2].

Several RSV intervention products are currently in clinical development, and in 2023, both an RSV maternal vaccine and a long-acting single-dose monoclonal antibody (mAb) have received market approval. However, due to limited resources, maternal vaccination and mAb access in LMICs will require support from international partners[3]. Gavi, the Vaccine Alliance, is an international organization established with the goal of creating equal access to new and underused vaccines for those living in LMICs. To increase the accessibility of RSV prevention methods worldwide, Gavi has included both maternal vaccination and monoclonal antibodies for the prevention of RSV as one of the six priorities in their Vaccine Investment Strategy (VIS) for the 2021–2025[4,5]. Every 5 years, the VIS sets new priorities for Gavi's vaccine support programmes based on impact, cost, value and programmatic feasibility of underused or new vaccines of highest relevance to Gavi-eligible countries[6]. In June 2024, the Gavi Board will take a final decision to inform Gavi investments between 2026–2030.

As both maternal vaccination and RSV monoclonal antibodies are characterized by a temporary protection profile, a good understanding of the age distribution of RSV-related deaths in Gavi-eligible countries will contribute to inform on impact and cost-effectiveness of RSV prophylaxis, thereby aiding Gavi in their decision-making process. The most commonly used estimates for the RSV-related mortality age-distribution in LMICs originate from a systematic review[1,7]. This study relied on a limited number of studies centered on disease incidence and mortality rates, which were often presented in broad age categories, producing indirect estimates. Furthermore, these estimates were not specific to Gavi-eligible countries. The WHO Strategic Advisory Group of Experts on Immunization (SAGE) underlined the key epidemiological gap of age-stratified data on RSV acute lower respiratory infection (ALRI) during the first months of life to inform the use and anticipated impact of prevention products[8,9]. Utilizing more detailed age-distributions enables a more precise estimation of the potential impact of RSV prevention strategies[10].

To fill the epidemiological gap of age-stratified disease burden data, the RSV Global Online Mortality Database (RSV GOLD) has been initiated in 2017, to elaborate on the clinical and sociodemographic profile of global RSV-related pediatric mortality. The first RSV GOLD retrospective case study was published in 2017 and included 117 cases from LMICs[11]. In 2021, a new retrospective case study was published comparing RSV-related infant community deaths with in-hospital deaths, including 829 deaths from LMICs[12]. However in that study, the majority of cases were from studies not eligible for Gavi, and the analysis on children below 2 years of age excluded 168 cases from community studies that only enrolled children up to 6 months of age.

In this study, we aim to investigate the characteristics of pediatric RSV mortality specifically in Gavi-eligible countries using all the data available from the RSV GOLD database. The detailed information on the age profile of RSV mortality will improve vaccine impact models and cost-effectiveness analysis, which are important for Gavi decision making.

## METHODS

*Study design and patients*

The RSV GOLD project is an ongoing global online mortality registry that collects individual patient data of children below 5 years of age who died with a laboratory-confirmed RSV infection after January 1st, 1995. Individual patient-level data are collected using an online questionnaire. Variables collected in the RSV GOLD database have been published previously[11,12]. In this article we categorize the data in the RSV GOLD database into two groups based on how the information was collected. The characteristics (country, setting, inclusion criteria, etc.) of all the studies included in the RSV GOLD database are presented in STable1. We refer to the first as Registry Mortality Data, which comprises data gathered through proactive outreach to researchers and physicians worldwide. These data were predominantly collected as part of *larger hospital-based surveillance studies*, mostly focused on respiratory infections in children. The sources are diverse, with variations in methodology and inclusion and exclusion criteria (see STable 1 for a detailed overview). The second group, referred to as Prospective Mortality Studies Data, consists of data from Bill & Melinda Gates Foundation (BMGF)-funded community mortality studies. These data were collected through *prospective hospital and community-based mortality surveillance*. Investigators of Bill & Melinda Gates Foundation (BMGF)-funded community mortality studies were specifically asked to share data collected up until March 2nd, 2021, as described previously in [12]. Two community studies (the Zambia Pertussis RSV Infant Mortality Estimation Study (Z-PRIME), and the Pakistan

Community Mortality studies) included children younger than 6 months of age; other studies recruited children up until at least 12 months of age.

All data were thoroughly reviewed by the RSV GOLD research team. Additional queries concerning inconsistencies or missing information have been verified through direct contact with the respective collaborator. In this analysis, RSV-related deaths above 2 years of age, nosocomial deaths, and deaths in high-income countries were excluded (**Figure 1**).



*Case definition*

For this analysis, we only included mortality cases from Gavi-eligible countries according to the classification from 2023[13]. As in our previous publications, we included any death with laboratory-confirmed RSV infection and did not require RSV to be the primary cause of death. If there was no information on where the RSV infection had been acquired and in the absence of nosocomial indications, we deemed the case community acquired.

The definition for a community death has been described previously[8,10,12]. If a child had not been admitted to hospital, we considered it as a community death. In case of missing data on the place of death, cases were classified as in-hospital deaths if the child had been admitted to hospital or if hospitalization status was not available. In case of missing data on hospitalization, we assumed the child had been hospitalized.

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 before 37 weeks' gestational age. If data for comorbidities and prematurity were not recorded, we assumed that the child was born healthy term.

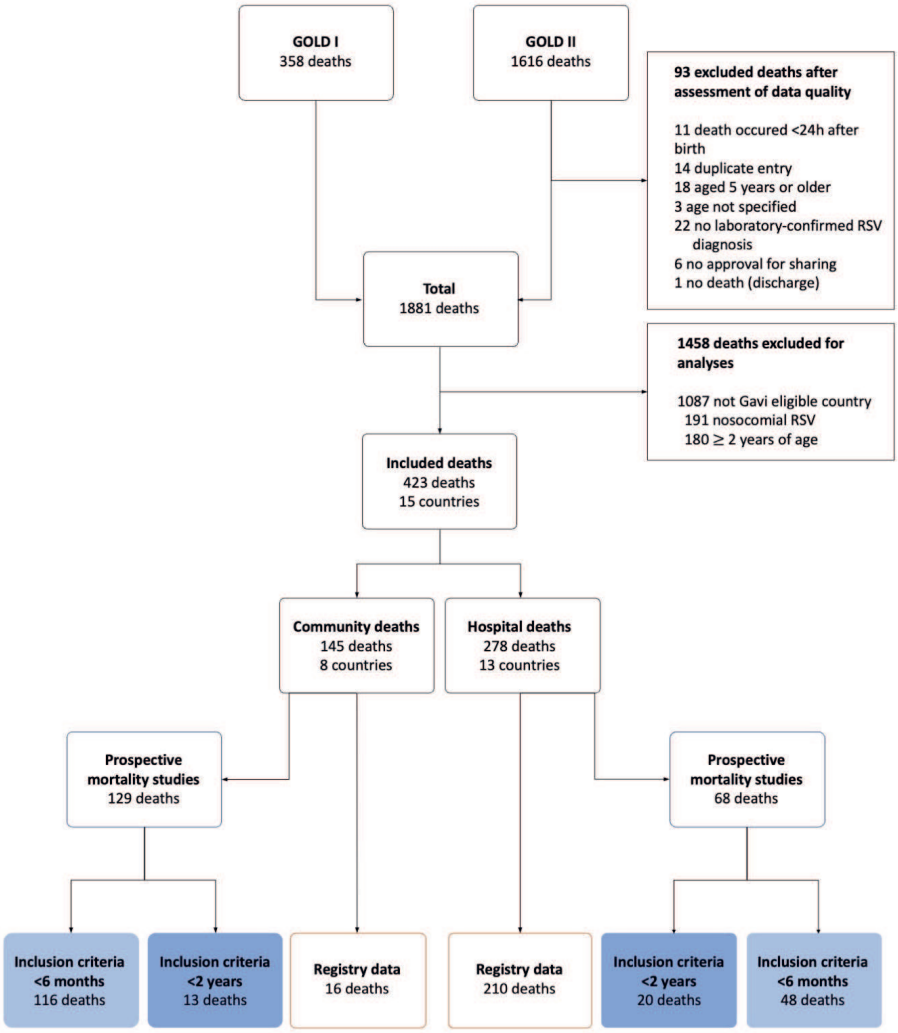



**Figure 1**. Flowchart of mortality cases included in this study. Flowchart shows children excluded via both data quality and per definition of study population. We incorporated the data from the <6 month and <24 months studies in our analysis using post-stratification weighting to adjust for overrepresentation. GOLD I: Pediatric deaths published as a retrospective case series from 1 November 2014 to 31 October 2015[1]. GOLD II includes pediatric deaths collected after this publication. Abbreviations: m, months; BMGF, Bill & Melinda Gates Foundation; GOLD, Global Online Mortality Database; RSV, respiratory syncytial virus; ZPRIME, Zambia Pertussis RSV Infant Mortality Estimation Study.

*Post-stratification weighting*

In the RSV GOLD database, two prospective community mortality studies (Z-PRIME and the Pakistan Community Mortality studies) included only children younger than 6 months of age, while the vast majority of the registry mortality data included data until at least 2 years of age (STable 1). To address a potential issue of over- or under-representation of particular age groups in our analysis, we employed post-stratification weighting. We adjusted the weights of undersampled and oversampled subpopulations with the goal of making the overall sample more representative of the true underlying population. The weights were calculated based on the demographic characteristics of the known population, to which we refer as the population weights. In our case, the oversampled subpopulation was the group of children in Gavi-eligible countries who died with RSV before 6 months of age. We therefore needed to adjust the proportion of children in Gavi-eligible countries dying with RSV before 6 months of age and the proportion of children dying with RSV at 6 months or older. In order to estimate population weights based on our available data, we utilized two distinct approaches referred to as the Complete Data Model and the Prospective Data Model. Without a compelling reason to favor one model over the other, we examined the results of both models side by side.

In short, the Complete Data Model (M1) includes both the Registry Mortality Data and the Prospective Mortality Studies Data, whereas the Prospective Data Model (M2) includes only the Prospective Mortality Studies Data (**Figure 2**). A complete illustrative comparison depicting the disparities between the Prospective Data Model and the Complete Data Model is presented in SFigure 1. More details about the models are described in Supplemental Methods.

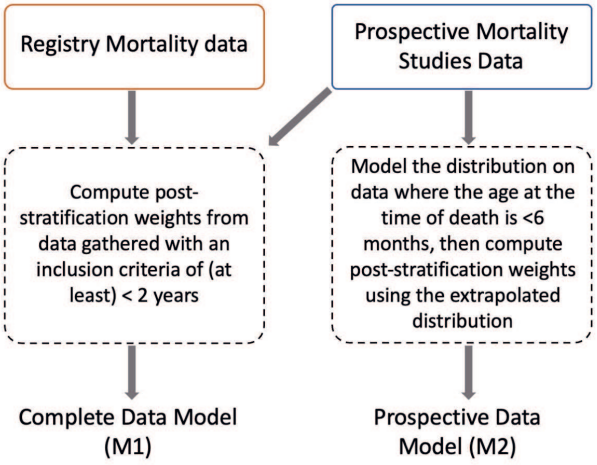



**Figure 2**. Visual Summary of the difference between the Complete Data Model (M1) and the Prospective Data Model (M2). See SFigure 1 for a more detailed version.

*Statistical analysis*

We report the mean age at time of death, the median age at time of death (i.e. the age at which half of the deaths among those under 2 years old had already taken place), and the peak age at time of death (i.e. the age at which RSV-related mortality reaches its peak). To quantify the proportion of cases in age groups, which is relevant for developing prophylactics, we calculated the proportion of cases below 1, 3 and 6 months of age.

For the other clinical features of interest, we calculated descriptive statistics, including measures such as mean, standard deviation, median, mode and interquartile range. Categorical variables were presented as frequencies and percentages.

We used a general linear model with the “glm” function in R to investigate the relationship between the place of death (in-hospital or community) and the data collection methodology (registry or prospective) and the age at time of death.

## RESULTS

*Study population*

We included 423 pediatric deaths under 2 years of age from 15 Gavi-eligible countries classified as LMIC according to the World Bank income group classification of 2023. Of these, 145 deaths occurred in the community (**Figure 1**). The Registry Mortality Data consisted of 226 deaths (**Figure 1**) from 13 different countries (**Figure 3A**), of which 16 were community mortality cases and 210 were in-hospital mortality cases. Place of death was missing for 35 cases, hospitalization was missing for 2 cases.



RSV diagnosis was established most often by PCR (76%). PCR was mostly used for children who died after 2005. The age at time of death distribution for the Registry Mortality Data is plotted in **Figure 4A**. The Prospective Mortality Studies Data consisted of 197 deaths (**Figure 1**) from 7 different countries (**Figure 3B**), of which 129 were community mortality cases and 68 were in-hospital mortality cases. RSV diagnosis was established by PCR for all these cases. Most deaths were from Zambia (76%, 150/197). The age at time of death distribution for the Prospective Mortality Studies Data is plotted in Figure **4B**.

*Pooling hospital and community cases*

Given the limited number of community mortality cases in the registry data, and the limited number of in-hospital mortality cases in the prospective data, we conducted a permutation test to determine whether it is appropriate to combine the hospital and community mortality cases for the analyses. We computed the difference in medians through 1000 permutations, comparing permuted differences to the observed one to calculate a p-value. The test showed no significant median age difference between community and in-hospital mortality cases ($p = 0.69$ for the registry data and $p = 0.67$ for the prospective data below 6 months). We therefore concluded that these cases could be pooled in the main analysis. Similarly, we tested whether the observations below 6 months of age differed significantly between the registry and prospective group. The permutation test did not yield a statistically significant result ($p = 0.44$), indicating that pooling of the data was acceptable.

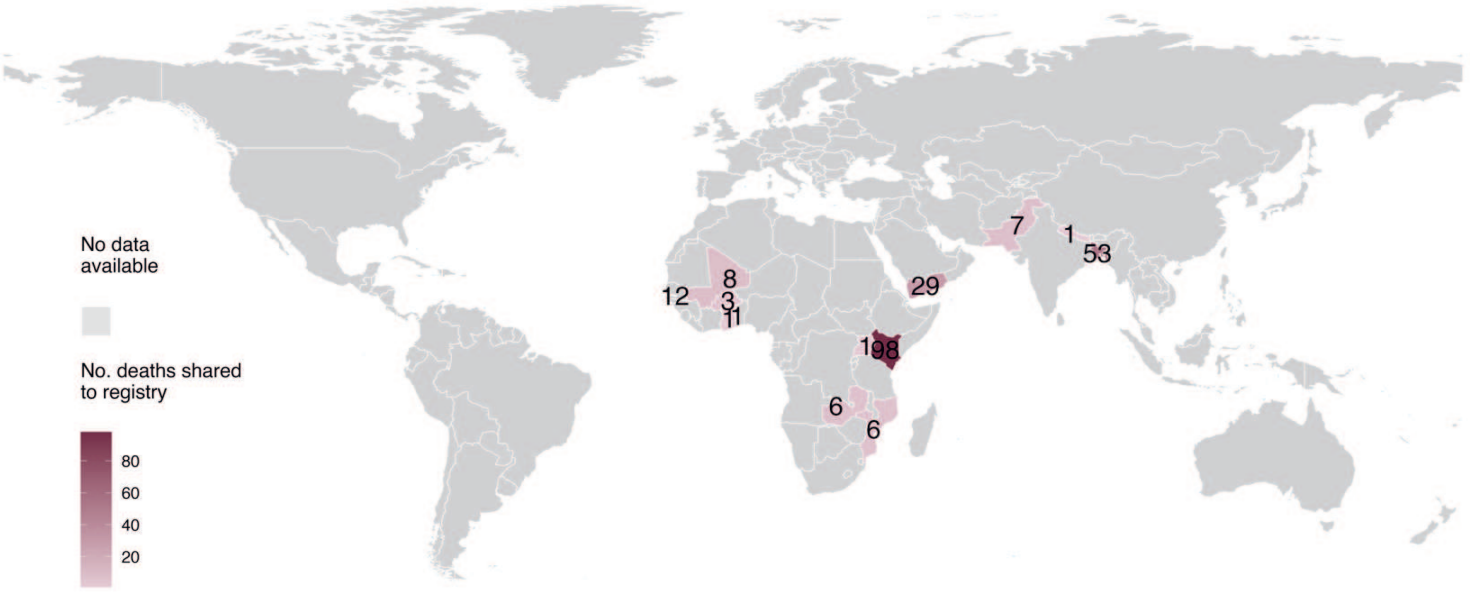



**Figure 3A:** Countries of origin of included children with RSV-related mortality for the Registry Mortality Data, reference data for M1. The color gradient indicates the number of deaths shared, with a darker color representing a larger number of deaths shared.

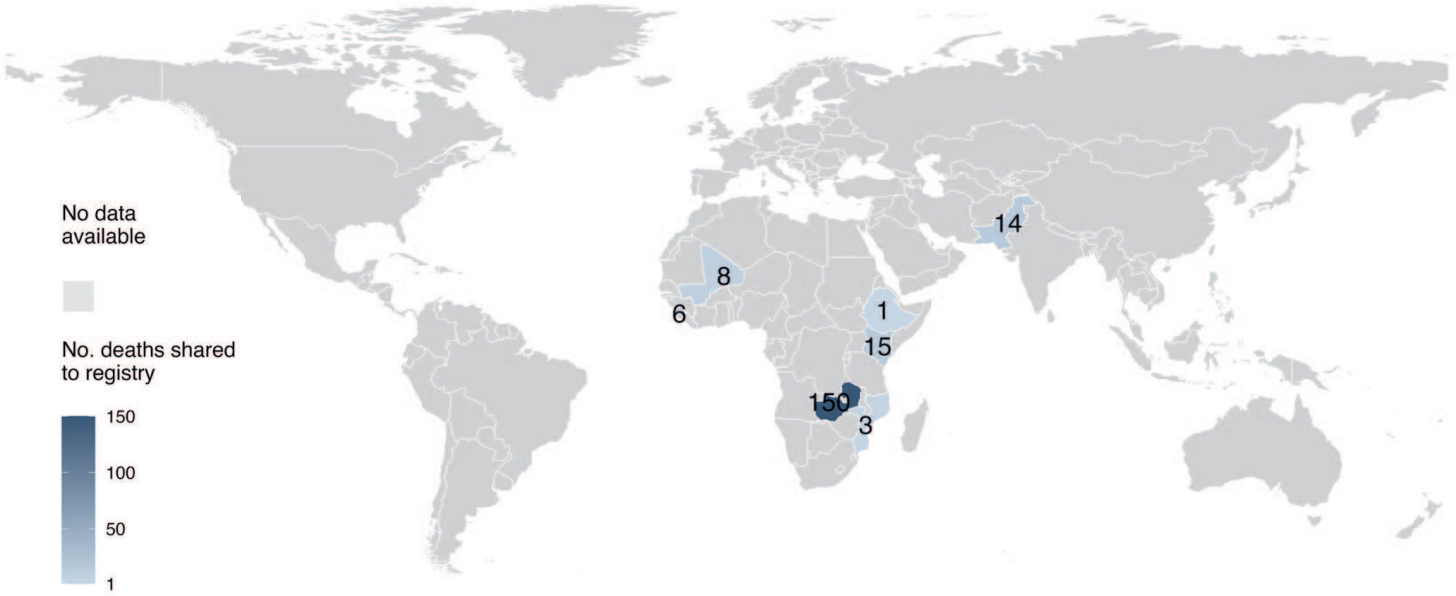
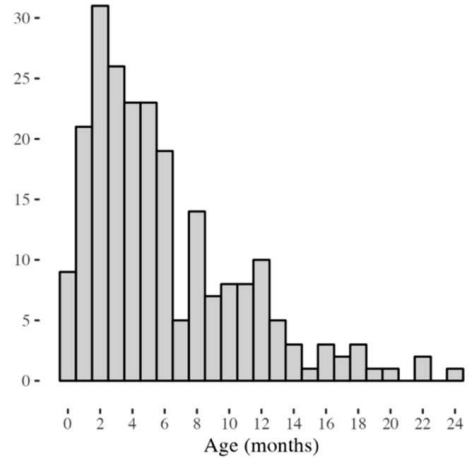



**Figure 3B:** Countries of origin of included children with RSV-related mortality for the Prospective Mortality Studies Data, reference data for M2. The color gradient indicates the number of deaths shared, with a darker color representing a larger number of deaths shared.

A




B

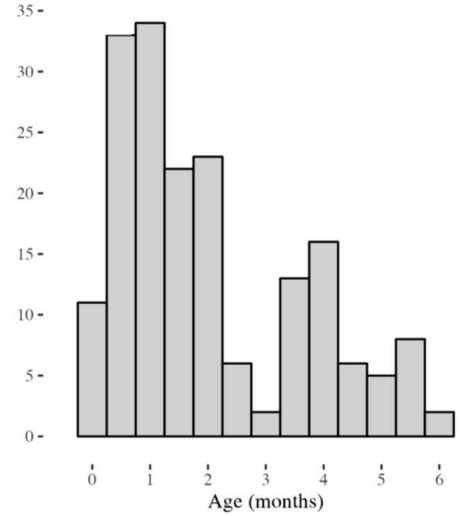



**Figure 4.** (**A**) Histogram of age at RSV-related death for children under 2 years in the Registry Mortality Data (reference data for M1). (**B**) Histogram of age at RSV-related death for children under 2 years in the Prospective Mortality Studies Data (reference data for M2). The histograms show the number of deaths (count) shared to the registry by age at death in months (rounded to the nearest integer).

*Post-stratification weights*

Complete Data Model (M1)

In this model, we made the assumption that using all available data, except for the Z-PRIME and Pakistan data, which had divergent age inclusion criteria, would provide us with the most accurate representation of the true population. This data encompasses observations across the entire age spectrum up to 2 years. Based on this data, we estimated the true proportion of mortality cases below 6 months of age to be 0.59. This corresponds to a weight of 0.788 for cases before 6 months of age and a weight of 1.633 for 6 months and older.

Prospective Data Model (M2)

In this model, we assumed that the subset of data obtained from the Prospective Community Mortality Studies is the most informative as this data was collected through active surveillance. We fitted a truncated Burr distribution (type XII) to the data below 6 months of age. The Burr distribution (Burr type XII) has three parameters[14]; scale, shape 1 and shape 2. The cumulative distribution function of the Burr distribution (for *x* weeks of age) is $F(x) = 1 - [1 + \left(\frac{x}{\text{scale}}\right)^{\text{shape 1}}]^{-\text{shape 2}}$. Our analysis identified the most suitable fit with parameters Burr(scale = 11.0, shape1= 1.2, shape 2 = 0.9) (**Figure 5B**). Based on the extrapolated distribution, we estimated the proportion of mortality cases below 6 months of age to be 0.77. This corresponds to a weight of 0.839 for cases before 6 months of age and a weight of 2.817 for 6 months and older.

To test the fit of the truncated distribution, we performed both a visual inspection (**Figure 5B**) and a simulation test. We simulated data from the truncated distribution with the same sample size as the observed sample and calculated the median age. This was repeated a 1000 times. The median from the observed sample was compared with the distribution of medians from the simulated samples and a corresponding p-value was calculated. The permutation test did not yield a statistically significant result (p = 0.99), indicating that there is no compelling evidence that the fitted distribution is not representative of the true underlying age distribution.

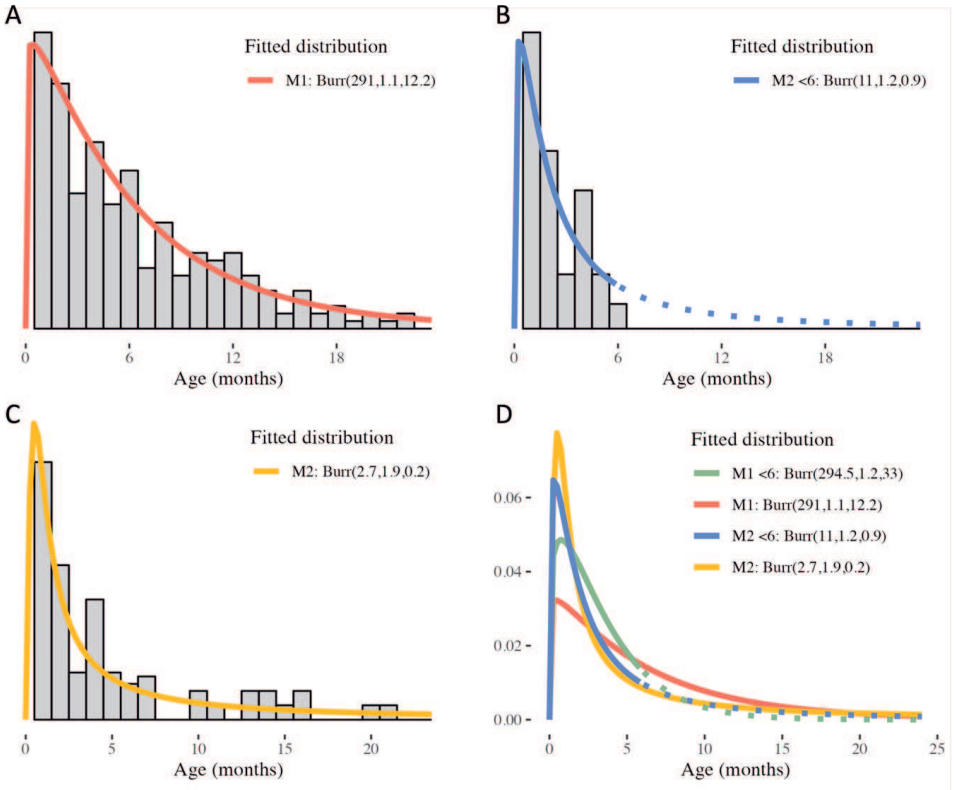



***Figure 5***. Fits of the Burr distribution to the age at RSV-related death data. The Burr distribution (Burr type XII) has three parameters: scale, shape 1 and shape 2. The fitted parameters are presented in the plot legend as Burr(scale, shape 1, shape 2). The cumulative distribution function of the Burr distribution (for x weeks of age) is $F(x) = 1 - [1 + \left(\frac{x}{\text{scale}}\right)^{\text{shape 1}}]^{-\text{shape 2}}$. (**A**) Weighted histogram (in grey) and fitted distribution (in red) for M1 under 2 years of age. (**B**) Data from the Prospective Community Mortality Studies below 6 months of age (in grey) and the corresponding fitted truncated distribution (in blue). (**C**) Weighted histogram (in grey) for M2 under 2 years of age and the fitted distribution (in yellow). (**D**) Comparison of the fitted distributions for M1 (in red the Burr-distribution fitted on the full dataset, in green the fitted Burr-distribution on data below 6 months of age only) and M2 (in yellow the Burr-distribution fitted on the weighted dataset, in blue the fitted Burr-distribution on data below 6 months of age only).

*Clinical characteristics*

The metrics of the age profile (mean, median, and peak age) are presented in **Table 1** for both the raw registry data and the raw prospective data (not adjusted for an overrepresentation of <6 months), and according to M1 and M2. Whereas the peak age of RSV-related mortality is consistent among the two models (driven by the prospective data, **Table 1**), M2 shows a younger age profile for the other metrics, with a median age difference of 2.2 months. Both models agree that the majority of RSV-related mortality cases from Gavi-eligible countries (59-77%) occur before 6 months of life.

Besides describing the age profile, our goal is to provide direct input for cost-effectiveness models. To accomplish this, we fitted a truncated Burr distribution to the M1 data for up to two years of age (**Figure 5A**). Given that, for M2, the Burr distribution is fitted using information from below six months of age (**Figure 5B**), we similarly utilized a truncated Burr distribution exclusively for the M1 data below six months. This approach ensures a fair comparison between the fitted distributions for both models and provides insight into the extent to which different age cut-offs impacted the fitted distribution. M2 yields a younger age-distribution compared to M1 (**Figure 5D**). When only data below 6 months of age is used to fit M1, the age-distribution is still considerably younger compared to that of M2.

The q-q plots show a reasonably close alignment between the quantiles of the observed data and those predicted by the fitted distributions (SFigure 3-6), except for the data fitted based on M2 up to two years of age. This is understandable, as the number of observations past 6 months are very limited in M2 and not evenly spread out (**Figure 5C**). A visual comparison suggests a favorable fit of the distributions to the empirical data (**Figure 5A-C**).

### Comorbidity and prematurity

Our estimations indicate that a minimum of 31% and 30% of mortality cases, for M1 and M2 respectively, had severe comorbidities (**Table 1**). Furthermore, based on M1 and M2, we calculated that a minimum of 9% and 10% of mortality cases, respectively, were born prematurely. Below 6 months of age, HIV/AIDS was the second most common reported comorbidity after congenital heart disease (STable 2). However, data on comorbidities and prematurity were often missing, especially for the Prospective Community Mortality Data cases (STable 3), limiting the power to analyze this characteristic. To address potential bias and test our assumption that children with missing data were born healthy term, we performed a sensitivity analysis excluding cases with missing data for prematurity or comorbidities. When excluding children with missing data for comorbidities and gestational age (STable 3), the percentage of children with comorbidities or prematurity more than doubled (to 59% and 26% for M1, and to 82% and 27% for M2, respectively).

### Community deaths vs in-hospital deaths

To address potential bias stemming from differences in data collection methodology (registry or prospective), a separate analysis was conducted for community and in-hospital deaths in both data collection approaches below 6 months of age (STable 4). Under 6 months of age, the observed age at time of death for in-hospital cases is older compared to community deaths. For both in-hospital cases and community deaths, the estimates for age at time of death are consistently older based on the Registry Mortality Data compared to the Prospective Community Mortality Data. We used a general linear model to compare the age at time of death (in months) for community deaths and in-hospital deaths below 6 months of age, while taking the differences in methodology into account. We found a significant effect of methodology on age at time of death, with the Prospective Community Mortality Studies resulting in lower estimates compared to the Registry Mortality Data ($b = -0.7$, $p = 0.002$), while the place of death (in community or in-hospital) showed no significant effect ($b = 0.2$, $p = 0.35$).

**Table 1.** Clinical characteristics of children under 5 years of age, who died with RSV in-hospital versus in the community in Gavi-eligible countries. Descriptive statistics (%, mean, medium, IQR) were calculated using survey weights, adjusting for the overrepresentation of studies with different age-related inclusion criteria. n is the reported frequency for a specific outcome in the dataset, N is the number of non-missing observations for the variable. n/N is the observed proportion in the database, not adjusted for survey weights.

| Clinical Characteristics | Registry data N = 226 | Unadjusted Prospective Data* N = 197 | Complete Data Model (M1) N = 423 | Prospective Data Model (M2) N = 197 |
|---|---|---|---|---|
| Sex, male, % (n) | 52 (117/226) | 48 (87/183) | 51 (210/412) | 47 (87/185) |
| Age at death, months, peak age (%) | 2 (14) | 1 (30) | 1 (16) | 1 (26) |
| Age at death, months, mean (SD) | 6.0 (4.8) | 2.9 (3.5) | 5.9 (5.2) | 4.4 (5.1) |
| Age at death, months, median (IQR) | 4.9 (2.2-8.0) | 1.7 (0.8-3.9) | 4.3 (1.8-8.9) | 2.1 (0.9-5.5) |
| Age <1 m at death, % (n) | 7.5 (17) | 30 (60) | 14 (61) | 26 (50) |
| Age <3 m at death, % (n) | 30 (68) | 66 (131) | 37 (157) | 56 (110) |
| Age <6 m at death, % (n) | 60 (136) | 92 (181) | 59 (250) | 77 (152) |
| Comorbidity, % (n) | 37 (83) | 18 (35) | 31 (133) | 30 (59) |
| Prematurity, % (n) | 8.4 (19) | 12 (23) | 9.0 (38) | 9.8 (19) |
| Hospitalized, % (n) | 96 (217) | 37 (73) | 73 (311) | 40 (79) |
| Gestational age (weeks) mean (SD) | 37.1 (4.5) | 35.9 (3.5) | 36.4 (4.1) | 37.0 (2.9) |
| n | 13 | 16 | 32 | 25 |
| Birth weight (kg) median (IQR) | 2.6 (1.8-3.0) | 2.8 (2.3-3.3) | 2.8 (2.1-3.2) | 2.9 (2.5-3.3) |
| n | 22 | 31 | 59 | 53 |
| Year of death, minimum - maximum | 1995-2022 | 2017-2021 | 1995 - 2022 | 2017-2021 |
| Not immunized, % (n/N) | 12 (8/69) | 53 (41/78) | 26 (39/152) | 39 (36/93) |
| Other children in household, % (n/N) | 82 (28/34) | 88 (29/33) | 86 (64/75) | 93 (44/47) |
| Mother uneducated, % (n/N) | 21 (8/39) | 11 (13/114) | 15 (20/130) | 11 (11/96) |
| Father uneducated, % (n/N) | 0 (0/21) | 4.0 (4/100) | 3.2 (3/98) | 4.0 (3/84) |

* Not adjusted for the overrepresentation of cases <6 months of age

## DISCUSSION

To the best of our knowledge, this is the first global mortality study characterizing children dying with RSV specifically in Gavi-eligible countries. We found a peak age of RSV-related mortality at 1 month of age and the majority of RSV-related mortality cases from Gavi-eligible countries occur before 3-6 months of life. Thus, we expect that implementing infant RSV immunization strategies, such as maternal vaccination or infant immunoprophylaxis, will have high impact on RSV-related mortality in Gavi-eligible countries.

Impact and cost-effectiveness studies are required to further evaluate health and economic impact of RSV interventions for Gavi eligible countries. To inform these studies, we reported the fitted age at time of death distributions. We utilized a Burr distribution to model our data, as this continuous probability distribution is commonly employed to describe the duration of survival. Furthermore, the Burr-distribution is used as input for the UNIVAC model (step 2 of inputs page)in [17]); a decision-support model with a universal framework for evaluating the potential impact and cost-effectiveness of different vaccines. An adaptation of the UNIVAC model allows the evaluation of RSV maternal vaccines and infant mAbs[16,17] and is therefore particularly useful in cost-effectiveness studies for RSV interventions.

We used two different models to estimate the age profile. The first model (M1) was based on all available data in the RSV GOLD database. The second model (M2) was based on only the data shared with the RSV GOLD project that were collected as part of the Prospective Community Mortality studies. Both M1 and M2 show a peak age of RSV-related mortality at 1 month of age. The majority of RSV-related mortality cases from Gavi-eligible countries occur before 6 months of life. According to M2, the majority of RSV-related mortality cases occur even before 3 months of life, suggesting a younger age-distribution compared to M1. The younger age profile of RSV mortality according to M2 is most in line with new available surveillance data. These contain thirty additional mortality cases from Gavi-eligible countries, gathered as part of the RSV GOLD ICU Network study, a recent active RSV surveillance study focusing on children under 2 years old, which have not been incorporated into the present analysis. The age profile of these cases is similar to that described in M2 (RSV GOLD ICU, manuscript submitted).

On the other hand, the age distribution derived from M1 aligns more closely with previously described age distributions in the context of cost-effectiveness analyses in LMICs (SFigure 2). In a cost-effectiveness study conducted in Vietnam[17], a Burr-distribution was fitted to severe RSV-ALRI hospital admission data below 2 years of age. This distribution corresponds to a median age of 35 weeks. In another study by Mahmud et al.[16], data from RSV hospital admission in six LMICs were analyzed as part

of their cost-effectiveness analysis. They identified the best fit for a Burr-distribution corresponding to a median age 23 weeks. Notably, their fitted age-distributions for severe RSV-ALRI exhibited significant variability across countries (see Figure S1 in [16].). In both cost-effectiveness studies, the age-distribution for mortality cases was assumed to follow the age-distribution for hospital admission. However, this could result in an overestimation of the age-distribution, as mortality cases are generally younger (unpublished data). This might explain why their estimates are notably older compared to our estimates.



For the first model (M1), it was assumed that using all available data except for the two community mortality studies, which had divergent age inclusion criteria, would provide us the most accurate representation of the true population. This assumption was based on two main factors: firstly, this data encompasses observations across the entire age spectrum up to 2 years, allowing for a comprehensive understanding of the age distribution below 2 years of age. Secondly, this is a relatively large and geographically diverse dataset. We acknowledge potential limitations in study methodology for the regular RSV GOLD registry cases; most of this data is gathered by our collaborators from hospital surveillance data or by systematically searching through hospital files. Heterogeneities in factors such as study setting, health-care access and seeking behaviour and eligibility for RSV testing could affect our estimates. For example, it has been speculated that younger children with RSV may present with nonspecific symptoms to the hospital and will therefore be overlooked[12]. Furthermore, children with reported severe comorbidities (who are generally older[11]) have better access to health care and health monitoring and therefore a higher probability of being tested and reported as RSV related death in the RSV GOLD database. Additionally, data pollution may arise due to the inclusion of children who died with severe comorbidities, where RSV was not a causal factor. These factors possibly explain the older age-distribution compared to M2.

For the second model, it was assumed that the subset of data obtained from the community mortality studies offers the most reliable depiction of the true population below 6 months of age. Unlike the regular GOLD registry cases, all these cases were collected through active surveillance. This active surveillance approach enhances the likelihood of capturing a comprehensive representation of the target population. An obvious limitation of this model is that the majority of the data originated from only one study site (Z-PRIME), and that it thereby makes the implicit assumption that the results of a single country are representative for all Gavi-eligible countries. Additionally, the reference data for this model only included children below 6 months of age. Therefore, M2 relies on the strong assumption that we can extrapolate the fitted distribution, obtained from data below 6 months, to up to 2 years of age.

Consistent with our previous analysis[12], we observed a lower median age at death in the community compared with in-hospital, although differences were not statistically significant when correcting for data collection methodology. Data collection methodology, on the other hand, was a significant predictor for age at time of death; with the Prospective Community Mortality Studies resulting in lower estimates compared to the Registry Mortality Data. This stresses the need for more high-quality prospectively collected mortality data from Gavi-eligible countries, to allow for more robust conclusions regarding the age profile of RSV mortality cases.

## CONCLUSION

We expect that implementing infant RSV immunization strategies, such as maternal vaccination or infant immunoprophylaxis, will have high impact on RSV-related mortality in Gavi-eligible countries. We further conclude that the potential biases in retrospective and surveillance data influencing the age profile of RSV mortality cases should be considered when performing cost-effectiveness analyses and making policy decisions. We underscore the importance of collecting more high-quality prospectively collected mortality data from Gavi-eligible countries.

## NOTES

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*Ethical approval*

Since solely secondary anonymous data were used in this study, the Medical Ethics Review Committee at the University Medical Centre Utrecht waived parental informed consent. However, adherence to local guidelines was encouraged and collaborators obtained ethical approval whenever necessary.

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*Role of the funding source*

The RSV GOLD study is funded by the BMGF, which had no role in the study design, data collection, analysis, and interpretation, or the writing of the article.

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# Part II

## Potential impact of maternal vaccination



"The art of simplicity is a puzzle of complexity"
- Douglas Harton

# Chapter 4

# Maternal vaccination against RSV can substantially reduce childhood mortality in low-income and middle-income countries: A mathematical modeling study

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## ABSTRACT

**Background**:

Respiratory syncytial virus (RSV) is a leading cause of childhood mortality in infants below 6 months of age. In low-income and middle-income countries (LMICs), the public health burden is substantial and resources are limited. It is critical to inform decision makers about effectiveness of new interventions.

**Methods**:

We developed a mathematical model where individual RSV subtype A (RSV-A) and B (RSV-B) maternally derived neutralizing titers were predicted at time of birth after maternal vaccination with the RSV prefusion F protein-based (RSVpreF) vaccine. We estimated the subsequent duration of vaccine-induced immunity and compared this to the age at time of death distribution in the RSV GOLD Mortality Database to predict the potential impact of maternal vaccination on RSV-related childhood mortality. We used country-specific timing of antenatal care visits distributions and mortality estimates to make country-specific predictions for number of cases averted.

**Findings**:

The model predicts that on average a neonate born at 40 weeks gestational age will be protected between 6 and 7 months from RSV-A and approximately 5 months from RSV-B related mortality. The optimal timing of vaccination for RSVpreF vaccine would be early in pregnancy. We estimated the potential impact of RSV-related mortality for in-hospital and out-of-hospital cases in LMICs and predicted that in 51 GAVI-eligible countries maternal vaccination could avert between 55% and 63% of the RSV-related in-hospital mortality cases below 6 months of age.

**Interpretation**:

We show that maternal vaccination could substantially decrease RSV-A and RSV-B related in- hospital and out-of-hospital mortality in LMICs in the first 6 months of life.

## INTRODUCTION

Respiratory syncytial virus (RSV) is a leading cause of childhood morbidity and mortality in infants below 5 years of age [1]. RSV-associated acute lower respiratory infections are responsible for worldwide 3.6 million hospital admissions and 26.300 in-hospital deaths annually. The vast majority (97%) of these RSV-attributable deaths occur in low-income and middle-income countries (LMICs), and almost half of these RSV-attributable deaths occur in the first 6 months of life [1]. Furthermore, it is estimated that for every in-hospital RSV-attributable death across all age groups, there are three out-of-hospital RSV-attributable deaths [1].

Multiple RSV interventions are currently in development [2]. Active vaccination will not protect the youngest infants, among whom the burden is the highest, since it will likely require several doses to elicit a robust vaccine-derived immune response [3]. Passive vaccination, via administration of monoclonal antibodies or maternal vaccination will provide only temporary protection [4]. Maternal immunization strategies aim to protect very young infants by transferring antibodies across the placenta from mother to fetus [5]. Maternal vaccines are in late stage of clinical development, and are anticipated to become available in the coming years [6].



In LMICs, the public health burden is substantial and resources are limited. Thus, it is critical that governments and large funding organizations are informed about effectiveness of new interventions so that interventions that have the highest impact and best value for money can be prioritized [3]. Seasonal dosing administration approaches in places with clear seasonality are considered to enhance the cost-effectiveness of maternal vaccination [4]. To optimize the RSV vaccination strategy, it is essential to know duration of protection by maternal vaccination.

The maternal vaccine known as RSVpreF, marketed under the brand name Abrysvo, has recently received approval from the European Medicines Agency (EMA) and could soon be available [7]. The corresponding phase 3 trial was conducted in 18 countries and included healthy pregnant women, 49 years of age or younger, at 24 through 36 weeks' gestation on the day of planned injection [6]. While a vaccine's efficacy is measured in a controlled clinical trial before approval, clinical trials alone will provide insufficient information to extrapolate vaccine efficacy to different populations due to the diversity in epidemiological settings in which preventive strategies could be applied [3]. Furthermore, the efficacy of maternal vaccines in specific risk groups, such as prematurely born infants, remain understudied in clinical trials due to the lack of statistical power [8]. Mathematical models represent a solution for studying the potential impact of different preventive strategies, depending on various vaccine characteristics and setting-specific health system factors without greatly increasing the size of trials [3]. Vaccine effectiveness estimates feed into cost-effectiveness

analyses, used by policy makers to prioritize interventions that have the highest impact and best value for money. Mathematical modeling could contribute to predict vaccine cost-effectiveness and inform decision making for governments and large funding organizations such as Gavi, the vaccine alliance [5].

Gavi considered some of the RSV interventions under development, including both maternal vaccination and monoclonal antibodies in its 2018 vaccine investment strategy for the 2021-2025 funding period. Health impact defined by the number of cases and deaths averted was used as one of the main criteria for prioritizing interventions [9]. There is however a paucity of data in LMICs on hospitalization and mortality in the current RSV modeling literature, specifically lacking geographical coverage [3]. In addition, out-of-hospital mortality has not been fully evaluated in the literature on RSV in LMICs due to a lack of data, despite the high burden of RSV morbidity in the population.

The present study predicts country-specific impact of maternal vaccination in LMICs on both in-hospital and out-of-hospital mortality. We show that our mathematical model provides a useful tool to make subgroup-specific predictions for future vaccine impact and to explore the impact of timing of vaccination and gestational age at time of birth on vaccine efficacy.

## METHODS

*Mathematical model*

As described previously [10], individual antibody titers of mother and infant pairs were modelled over time. We have made a few improvements compared to the model previously published. We decided to parameterize our model on the RSV prefusion F protein-based (RSVpreF) maternal vaccine [11], as this vaccine might already be registered in 2023 [12]. As immunogenity data for RSVpreF is reported in RSV subtype A (RSV-A) and B (RSV-B) neutralizing titers, we parameterized our model on RSV-A and RSV-B neutralizing antibodies. Furthermore, we assumed a peak in neutralizing activity in the mothers' serum 14 days post vaccination [13, 14, 15], in contrast with the assumed peak after 21 days in ref [10]. Lastly, in contrast to [10], we added maternal antibody decline during pregnancy in the model to match the phase 1/2 RSV RSVpreF trial data that is currently under development [16, 11]. The decline of maternal antibody levels after vaccination has also been observed in the mothers in the phase 3 Novavax maternal vaccine trial [17].

We hence assumed that maternal RSV-A and RSV-B neutralizing antibody titers increase exponentially from 7 days after vaccination, until they reach a peak at 14 days. After 14 days, maternal antibody levels decline exponentially. The maternal antibody titers are described in Equation 1. Where $t$ is the number of days since vaccination, $a_{m_0}$ is the maternal 50% neutralizing antibody titer (geometric mean titer level needed to prevent 50% of infections) before vaccination and $f$ the fold increase in neutralizing antibody titers 14 days post vaccination.

$$a_m(t) = \begin{cases} a_{m_0} & \text{if } t < 7 \\ a_{m_0} * e^{(t-7)\log(f)/7} & \text{if } t \leq 7 \leq 14 \\ a_{m_0} * f * \frac{1}{2}^{(t-14)/t_{M_{\frac{1}{2}}}} & \text{if } t < 14 \end{cases} \quad (1)$$

The foetus-to-mother antibody transfer function was modelled as described previously [10], such that the neonatal antibody titers ($a_n$) at gestational age at time of birth in days ($t_b$) depend on both the antibody transfer function $r(t_b)$ and maternal antibody titers, $a_m(t)$, as follows:

$$a_n\,(t_b, t) = r(t_b) * a_m(t) \quad (2)$$

Using the individual antibody titers of mother-fetus pairs during pregnancy, we predicted the number of days protected (which we refer to as duration of vaccine-induced immunity) from RSV-related death ($T_{prot}$) as described in Equation 3.

Where $t_{N_{\frac{1}{2}}}$ is the half-life in neonates in days and $A_{prot}$ is the protection threshold in 50% neutralizing antibody geometric mean titer.

$$T_{prot}(t_b, t) = \frac{t_{N_{\frac{1}{2}}} * \ln\left(\frac{A_{prot}}{a_n(t_b, t)}\right)}{-\ln(2)} \quad (3)$$

Parameter values were fixed according to the published phase 1/2 RSVpreF vaccine trial [11] and are reported in **Table 1**. We estimated fold increase at 14 days post vaccination and half-life of vaccine induced antibodies in the mother based on the reported RSV-A and RSV-B neutralizing geometric mean fold rises post-vaccination in ref [11] (See appendix S2 for more details).

Data on the decay of passively transferred antibodies are not yet available for the RSVpreF vaccine [18]. Therefore, we used data on the half-life in neonates of passively

transferred RSV-A and RSV-B neutralizing antibodies in the phase 2 RSV F vaccine trial [19], which is in close agreement with reported values in previous clinical studies [10]. We assumed that RSV neutralizing titers corresponding to palivizumab serum titers, equal or above 100 µg/ml could avert (or at least postpone) RSV-related mortality [11].

**Table 1**. Parameter values of the model.

| Parameter | Abbreviation | RSV-A | RSV-B | Source |
|---|---|---|---|---|
| Prevaccination antibody titers (50% neutralizing GMT) | $a_{m_0}$ | 1434 | 1366 | [9] |
| Fold increase 14 days post vaccination* | $f$ | 12.6 | 14.7 | [9] |
| Half-life of maternal antibodies* (days) | $t_{M_{\frac{1}{2}}}$ | 274.5 | 229.5 | [9] |
| Half-life of neonatal antibodies (days) | $t_{N_{\frac{1}{2}}}$ | 45.4 | 39.3 | [17] |
| Protection threshold (50% neutralizing GMT) | $A_{prot}$ | 903 | 2069 | [9] |

GMT = geometric mean titer, * = estimated based on available data in source

*RSV Gold database*

We made use of the RSV Global online Mortality Database [20] for predicting the potential impact of maternal vaccination on RSV-related childhood mortality. In short, RSV GOLD is an ongoing global online registry for infants under the age of 5 years who died with laboratory-confirmed RSV infection after January 1, 1995 [20]. Age at time of death is reported in days or in months by the collaborators. The variables collected in the RSV GOLD database and method of data collection have been published previously [20, 21, 22]. Two community studies who shared data with the RSV GOLD database only included children below 6 months of age [22]. To prevent selection bias in the age distribution, we included all in-hospital deaths and out-of-hospital deaths from infants in LMICs who died before 6 months of age in this study. This subset comprises 473 in-hospital deaths and 156 in-community deaths. Table S2 presents the clinical characteristics of the infants in these respective groups. Additionally, Figure S4b displays world maps illustrating the distribution of cases from various countries. The data from this subset has been published previously [22]. We decided to simulate data distributions to create more representative data. We simulated 1000 age at time of death distributions for both out-of-hospital deaths and in-hospital deaths, see appendix S1 for more details.

*Predicting vaccine efficacy*

We define vaccine efficacy (*E*) as the fraction of the number of deaths (*N*) in the dataset that could have been averted, had the mothers been vaccinated. The simplest approach would be to compare the observed time of death (*Tdeath*) with the estimated duration of vaccine-induced immunity ($Tprot$) had the mother been vaccinated during pregnancy:

$$E = \frac{1}{N}\sum_{i=1}^{N} f(Tprot_i) \quad (4)$$



Where

$$f(Tprot_i)\begin{cases} 0, \text{ if } Tprot_i < Tdeath_i \\ 1, \text{ if } Tprot_i \geq Tdeath_i \end{cases}$$

However, due to missing data on gestational age and potential bias in the reported age at time of death, we used simulated data distributions, to create more representative data instead of individual observations. To save computational time, we directly compare the estimated duration of vaccine-induced immunity with the simulated age at time of death distributions. We thereby estimate the probability that RSV-related mortality would have been averted ($p_{averted}$) by maternal vaccination, depending on gestational age at time of vaccination ($t_v$) and gestational age at time of birth ($t_b$). The probability that *T prot* ≥ *Tdeath* is estimated by taking the average area under the age at time of death curve, as follows:

$$p_{averted}(t_v, t_b) = \frac{1}{N_{agedist}} \sum_{j=1}^{N_{agedist}} \int_0^{T_{prot}} f_j(x)dx \quad (5)$$

where $N_{agedist}$ is the number of simulated age distributions and $f_j$ the function for the age at time of death distribution. We weighted the probability estimates $p_{averted}(t_v, t_b)$ by the estimated probability mass function (Figure S6b) for gestational age at time of birth as follows:

$$p_{weigthed}(t_v) = \frac{p_{averted}(t_v, t_b) * w(t_b)}{\sum w(t_b)} \quad (6)$$

where $\frac{w(t_b)}{\sum w(t_b)}$ represent the estimated probability that $t_b$ occurs in the population. This yields the estimated vaccine impact in the population, in terms of mortality cases averted, given the assumed timing of vaccination.

To evaluate the effect of timing of maternal vaccination we considered an administration window for maternal vaccination between 24 and 36 weeks of gestation, as suggested in the current guidelines of the Phase 3 RSVpreF vaccine trial [23].

*Country-specific predictions on vaccine impact*

Antenatal care (ANC) visits could be an easy access to administer vaccines that require strict gestational age windows of administration [24]. We hence argue that the country-specific distribution of time of ANC visits within the administration window (between 24 and 36 weeks) could serve as a proxy for the likelihood distribution for timing of maternal vaccination. Data on the projected proportion of ANC attenders was provided by Baral. et al. [24]. Their paper includes a summary illustrating the variability of antenatal care (ANC) coverage estimates across different countries plot (Fig. 2 in [24]). They also provide proxy estimates pertaining to service availability and acceptance (S3 appendix in [24]).

To estimate the probability mass distribution for time of vaccination we summed the proportion of ANC attenders for visit 1, 2 and 3 together per week. The weekly total was divided by the summed total of the weekly proportions to create a probability mass distribution for time of vaccination ($g_{t_v}$).

$$g_{t_v}(t) = \frac{f_{V_1}(t) + f_{V_2}(t) + f_{V_3}(t)}{\sum_{24}^{36}(f_{V_1}(t) + f_{V_2}(t) + f_{V_3}(t))} \text{ for } 24 \leq t \leq 36$$

Where $f_{V_1}(t)$, $f_{V_2}(t)$ and $f_{V_3}(t)$ are the probability mass functions of ANC visit 1, visit 2, and visit 3 attendance.

Assuming that all eligible women receive a vaccine, which we define as achieving full vaccine coverage, we estimate the vaccine efficacy per country ($E_{country}$) by weighting the median percentage averted given time of vaccination by the probability mass distribution for each week within the administration window:

$$E_{country} = \sum_{24}^{36} P_{averted}(t_v) * g_{t_v}(t_v) \quad (7)$$

For a more realistic vaccine efficacy estimate, we multiplied $E_{country}$, by the estimated service availability and acceptance proxy reported by Baral et al. [24].

Lastly, country-specific RSV-related mortality burden estimates reported by Li et al. [25] were multiplied by the predicted percentage averted given realistic vaccine coverage, to predict the number of mortality cases averted yearly per country. For this step we only included countries where both the service availability and acceptance proxy was reported [24] and where age-specific mortality estimates were available [25]. As Li et al [25] only estimated country-specific in-hospital mortality rates, we multiplied the mortality burden estimates by three[1] for the out-of-hospital RSV-attributable deaths. All simulations and analyses were performed in R software (version 4.0.2.) [26].



## RESULTS

*Estimated duration of vaccine-induced immunity*

We estimated the neonatal RSV-A and RSV-B neutralizing antibody titers at time of birth (STable S3 and STable S4) for each possible combination of gestational age at time of vaccination and gestational age at time of birth (see Methods). Next, we calculated the subsequent duration of vaccine-induced immunity (**Figure 1**) for all these combinations.

The model predicts that on average a neonate born at 40 weeks gestational age will be protected for at least 196 days from RSV-A and 145 days from RSV-B related mortality (assuming that vaccination occurred at least 14 days prior to birth) (**Figure 1**). The prediction that the vaccine will provide longer protection from RSV-A compared to RSV-B is mostly driven by the higher protection threshold in relation to the prevaccination antibody titers for RSV-B.

For preterms (defined as born before 37 weeks' gestational age), the duration of vaccine-induced immunity depends strongly on gestational age at time of vaccination and gestational age at time of birth (**Figure 1**). The large range in duration of vaccine-induced immunity is mainly driven by a low antibody transfer early in pregnancy that quickly increases with gestational age. Lower antibody transfer at time of birth results in lower antibody titers and therefore a shorter duration of protection.

We applied our model in a situation without vaccination to estimate the duration of natural immunity. The model predicts no protection from RSV-A and RSV-B related mortality for preterm neonates born 35 weeks' gestational age or younger (Table S5). For full-term neonates, the protection ranges from 16 to 86 days (RSV-A) and from 0 to 29 days (RSV-B), dependent on gestational age at time of birth.

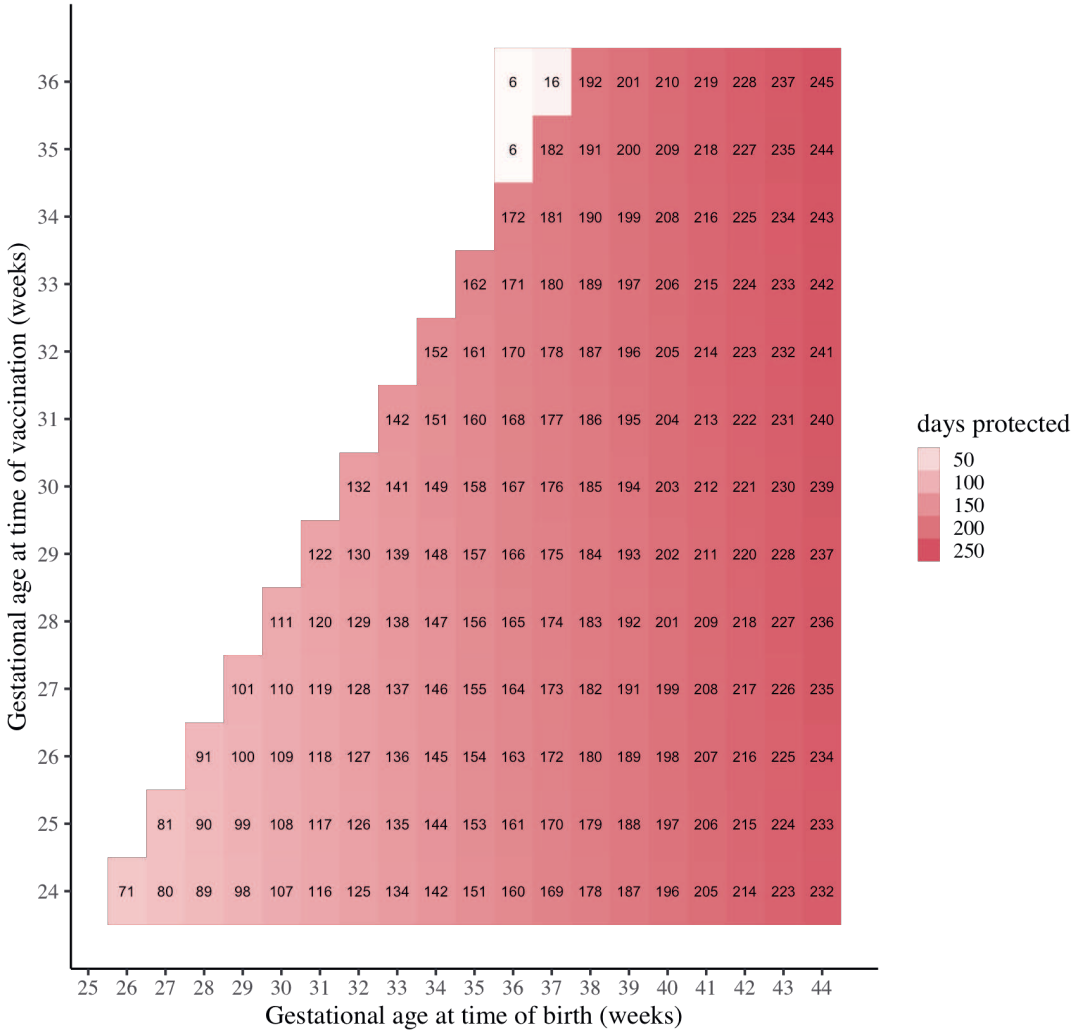



**Figure 1A**: The predicted duration of vaccine-induced immunity (in days) for RSV-A for each combination of gestational age at time of vaccination, and gestational age at time of birth.

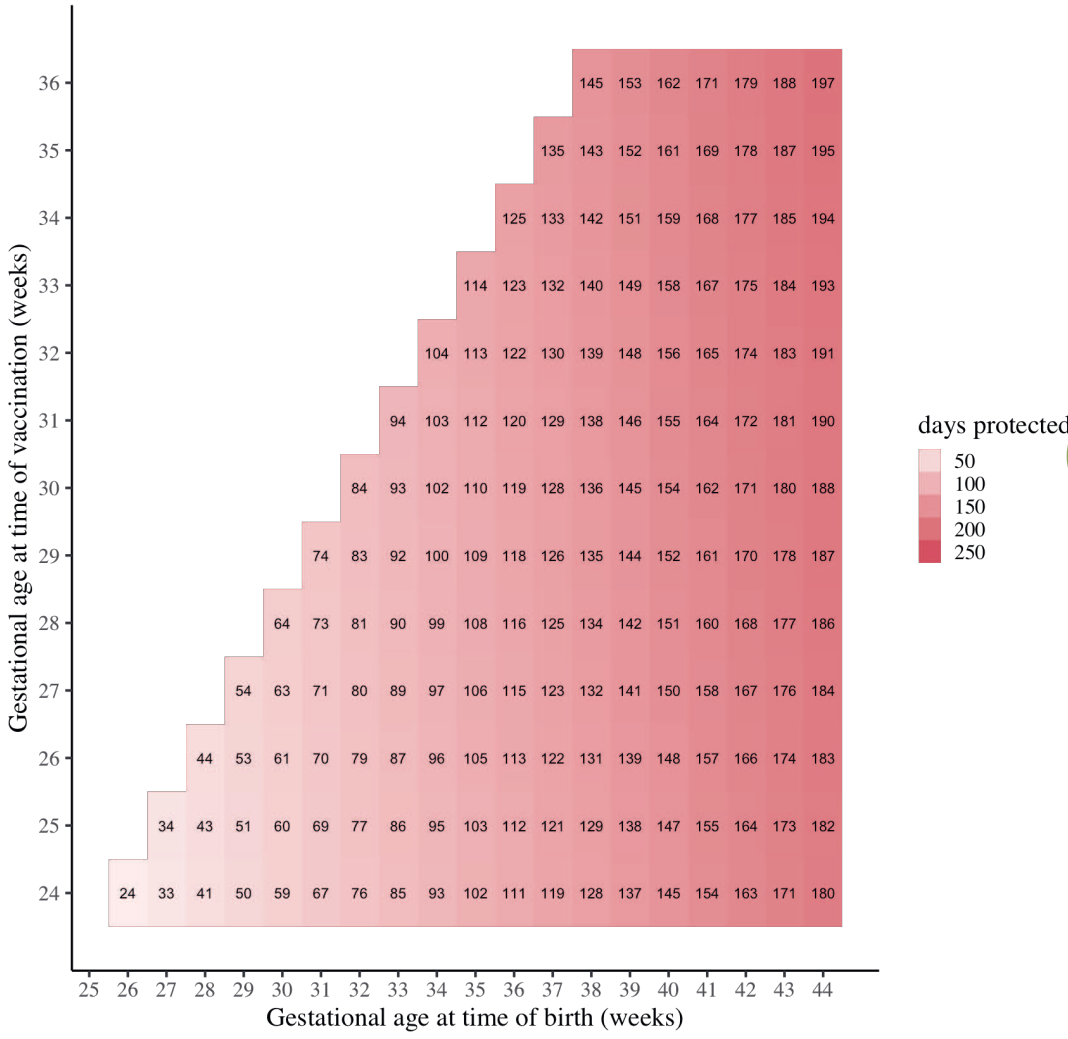



**Figure 1B**: The predicted duration of vaccine-induced immunity (in days) for RSV-B for each combination of gestational age at time of vaccination, and gestational age at time of birth.

*RSV GOLD database*

We applied our model to a subset of the RSV GOLD database [22], including all in-hospital deaths and out-of-hospital deaths from infants in LMICs who died before 6 months of age. The median age at time of death was 2.4 months for the in-hospital deaths, and 1.5 months for the out-of-hospital deaths (Table S2, Figure S4). The median reported gestational age was 38 weeks (IQR: 35, 39) for the in-hospital deaths, and 38 weeks (IQR: 38, 40) for the out-of-hospital deaths (Table S2, Figure S4). For the subset with the most reliable observations for gestational age (see Methods), the median gestational age was 38 weeks (IQR: 35, 39).

*Potential impact of maternal vaccination*

By considering the distribution of gestational age at the time of birth from the mortality cases in the RSV GOLD database, we made predictions on the proportion of infants who possess vaccine-induced immunity (i.e. their antibody concentration being above the protection threshold) at different ages (**Figure 2A** and **Figure 2B**). The fraction of prevented mortality is higher when vaccination takes place earlier during pregnancy as it is most likely that the vaccine will have less (or no) effect if the mother delivers soon after the vaccine administration or even before being vaccinated. On the other hand, if vaccination occurs too early during pregnancy, the prevented fraction begins to decline at a slightly younger age; because we assume that maternal antibody concentration starts to decrease 14 days after vaccination in the mother. Consequently, this leads to slightly lower antibody levels at the time of birth for full-term babies. Despite the slightly lower antibody levels at the time of birth for full-term babies, the advantages of vaccinating earlier in pregnancy far outweigh this downside.

Taking both the age at time of death and gestational age at time of birth distributions into account, we can predict vaccine impact in children younger than 6 months in LMICs. For RSV-A, the predicted vaccine-induced duration of protection exceeds 6 months for almost all combinations of gestational age at time of birth and gestational age at time of vaccination (**Figure 1**), hence providing almost full protection to both in-hospital and out-of-hospital deaths (**Figure 2**). For RSV-B this is not the case; as the predicted vaccine-induced duration of protection is shorter, the potential vaccine impact of the vaccine depends more on the specific age at time of death distribution, causing different impact estimates for in-hospital and out-of-hospital deaths.

*Optimal timing of vaccination*

The optimal timing of vaccination depends on the relation between gestational age and antibody transfer over the placenta, the gestational age at time of birth distribution in the population of interest, and the half-life of vaccine induced antibodies in the mother.

For RSV-A, the model predicts that vaccination at 25 weeks' (in-hospital mortality) and 24 weeks' (out-of-hospital mortality) gestational age would be optimal (**Figure 2C**, Table S6 and Table S7). For RSV-B, the model predicts that vaccination at 27 weeks' (in-hospital mortality) and 26 weeks' (out-of-hospital mortality) gestational age would be optimal (**Figure 2D**, Table S8 and Table S9).

The predicted optimal timing of vaccination is largely driven by the slow decay of vaccine induced antibodies in the mother for the RSVpreF vaccine (an estimated half-life of 274.5 for RSV-A and 229.5 for RSV-B). When we parameterized the model to the Novavax vaccine characteristics [17], with a half-life of vaccine induced antibodies in the mother of 145.6 days, we found that the optimal timing of vaccination was between 31 and 32 weeks gestational age (data not shown).



*Country-specific estimates for vaccination impact*

We assumed that antenatal care (ANC) visits could be used as opportunities to administer a maternal RSV vaccine. We used the country-specific distribution of time of ANC visits within the administration window as a proxy for the likelihood distribution for timing of maternal vaccination. The country-specific estimates for vaccine efficacy (**Figure 3**) were multiplied by the estimated service availability and acceptance proxy reported by Baral et al. [24]. This yields country-specific estimates for vaccine efficacy, assuming a more realistic vaccine coverage (**Figure 4** for in-hospital mortality and **Figure 5** for out-of-hospital mortality)

Lastly, we estimated for 51 GAVI-eligible countries the number of RSV-related mortality cases averted yearly, using the mortality estimates from Li et al. [25]. We estimated that for those 51 countries, between 17375 and 15062 in-hospital mortality cases total (estimate based on vaccine efficacy for RSV-A and RSV-B respectively) could be averted yearly (Table S11). This corresponds to a weighted average 63% of the RSV-A related deaths and 55% of the RSV-B related deaths averted in the selected countries.

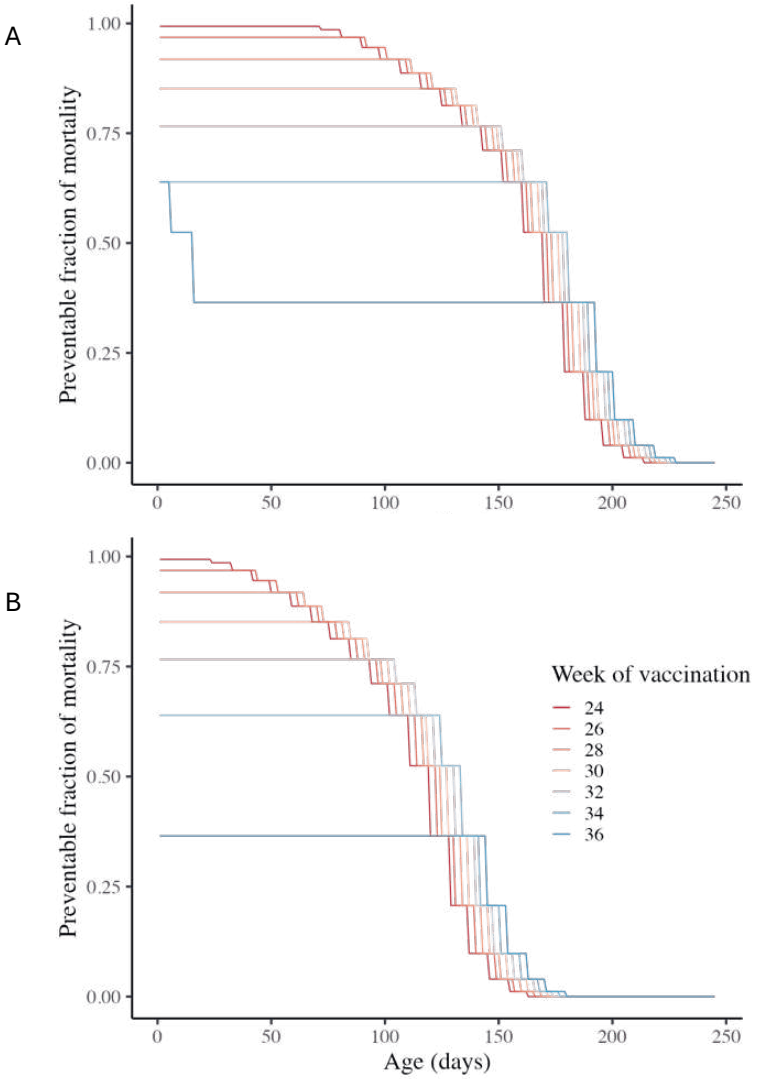
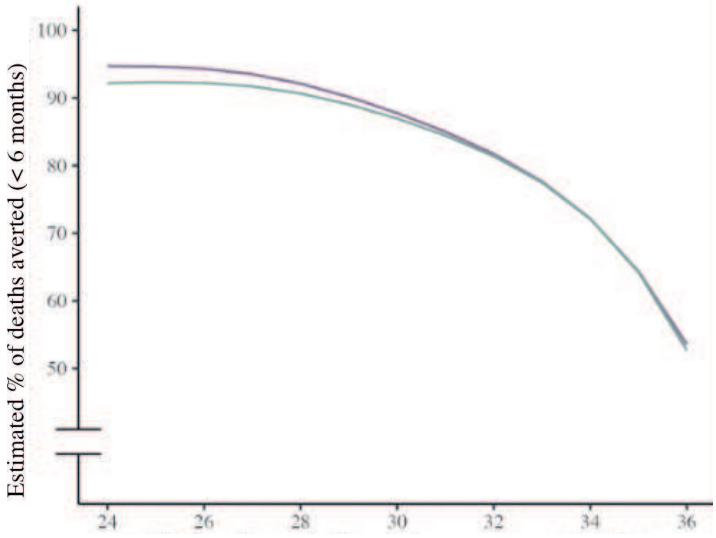



**Figure 2A and 2B**: Estimated potential impact of maternal vaccination on RSV-related mortality. Plot (**A**) and (**B**) show fraction of mortality cases for RSV-A (**A**) and RSV-B (**B**) that could be prevented for the different ages of the infants if all mothers would get vaccinated in a specific week (different weeks are shown in different colors). In other words, it shows the percentage of children who are born with vaccine-induced immunity and how this vaccine-induced immunity wanes over time. Here we see the trade-off between vaccinating earlier; increasing the number of individuals who are eligible to receive the vaccine, while the duration of protection will be slightly shorter.

C




D

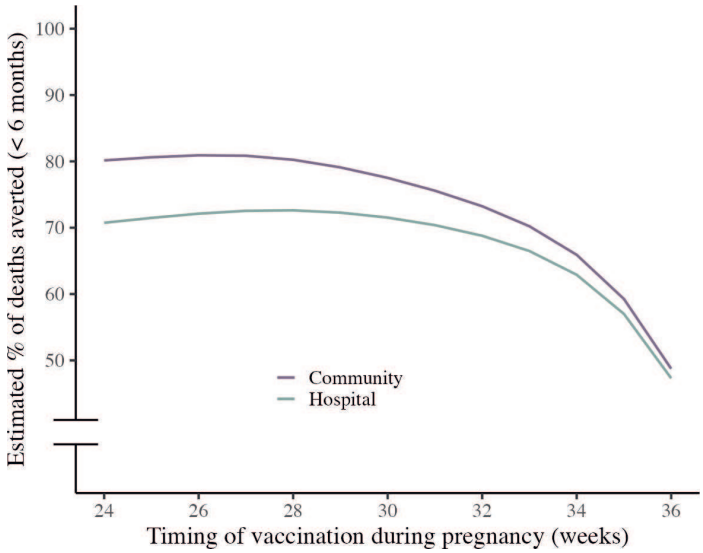



**Figure 2C and D**: Estimated potential impact of maternal vaccination on RSV-related mortality. Plot (**C**) and (**D**) show the estimates for the total percentage of RSV-A (**C**) and RSV-B (**D**) related mortality cases averted below 6 months of age for both out-of-hospital deaths (purple) and in-hospital deaths (green) given timing of vaccination, assuming that all mothers eligible for vaccination received a vaccine.

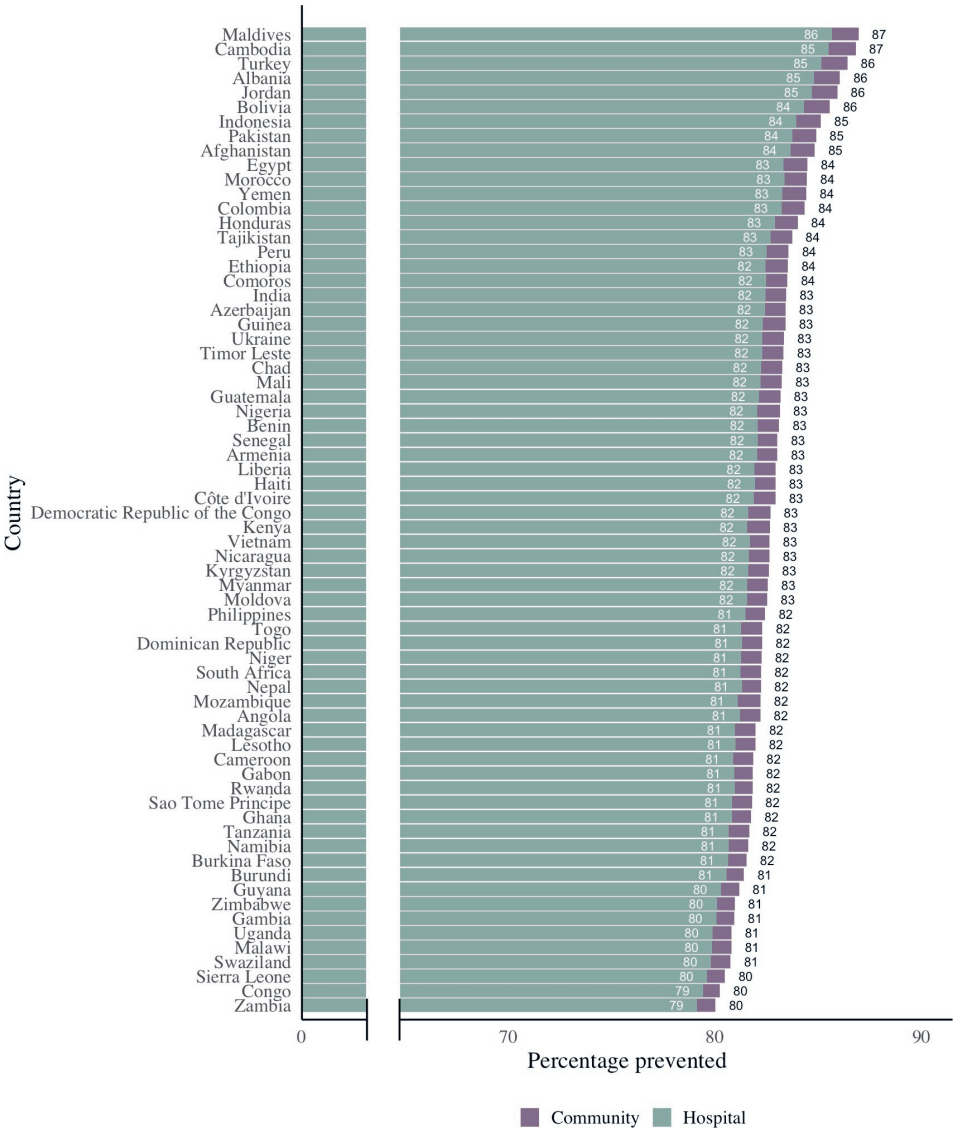



**Figure 3A**: Country-specific estimates for impact of maternal vaccination on RSV-A related mortality, assuming full vaccine coverage.

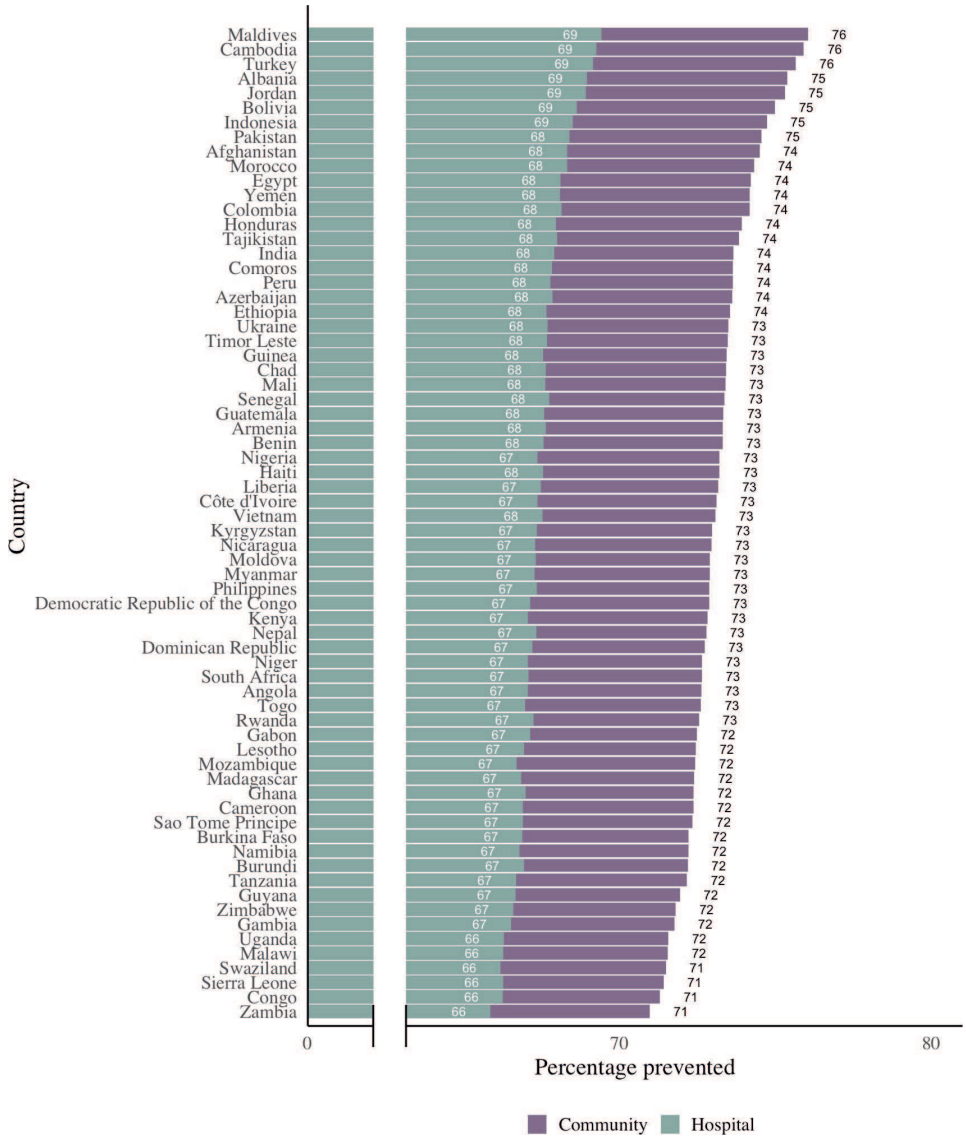



**Figure 3B**: Country-specific estimates for impact of maternal vaccination on RSV-B related mortality, assuming full vaccine coverage.

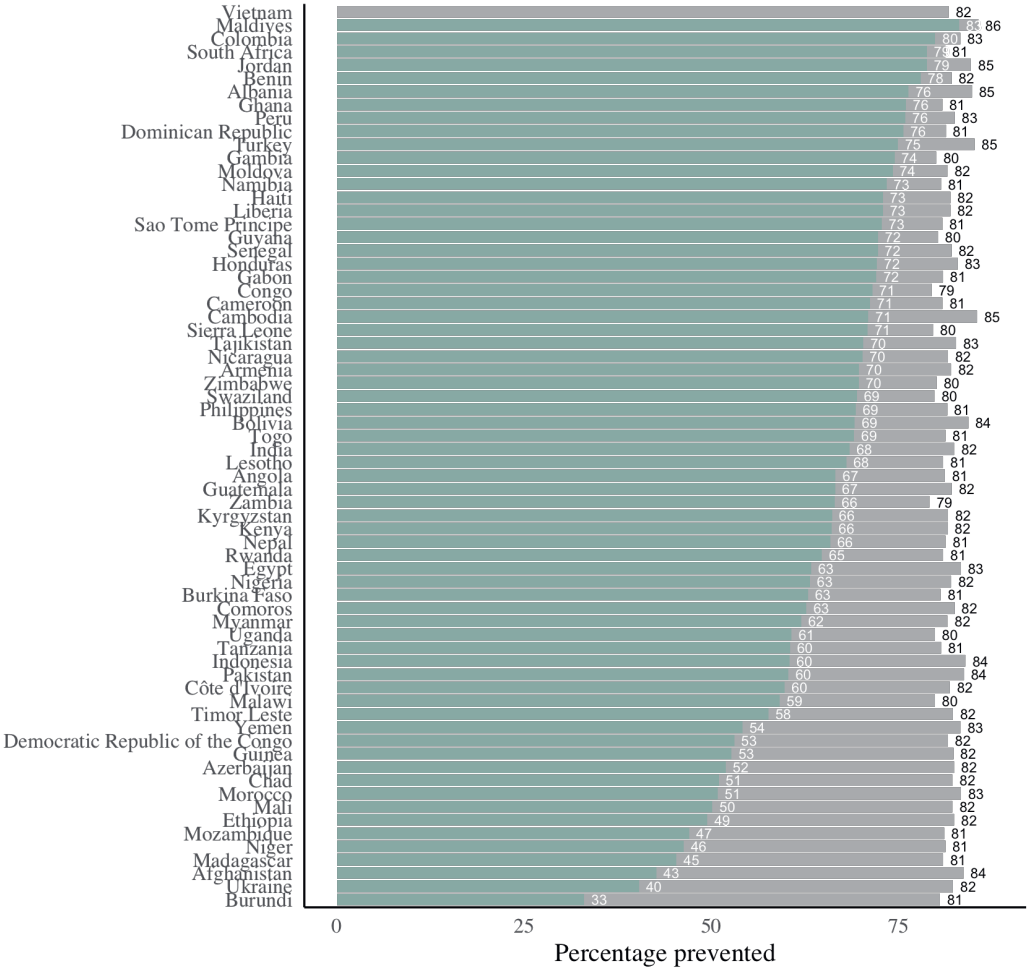



**Figure 4A**: Country-specific estimates for impact of maternal vaccination on RSV-A related in-hospital mortality, assuming full vaccine coverage (grey) and a realistic vaccine coverage (green).

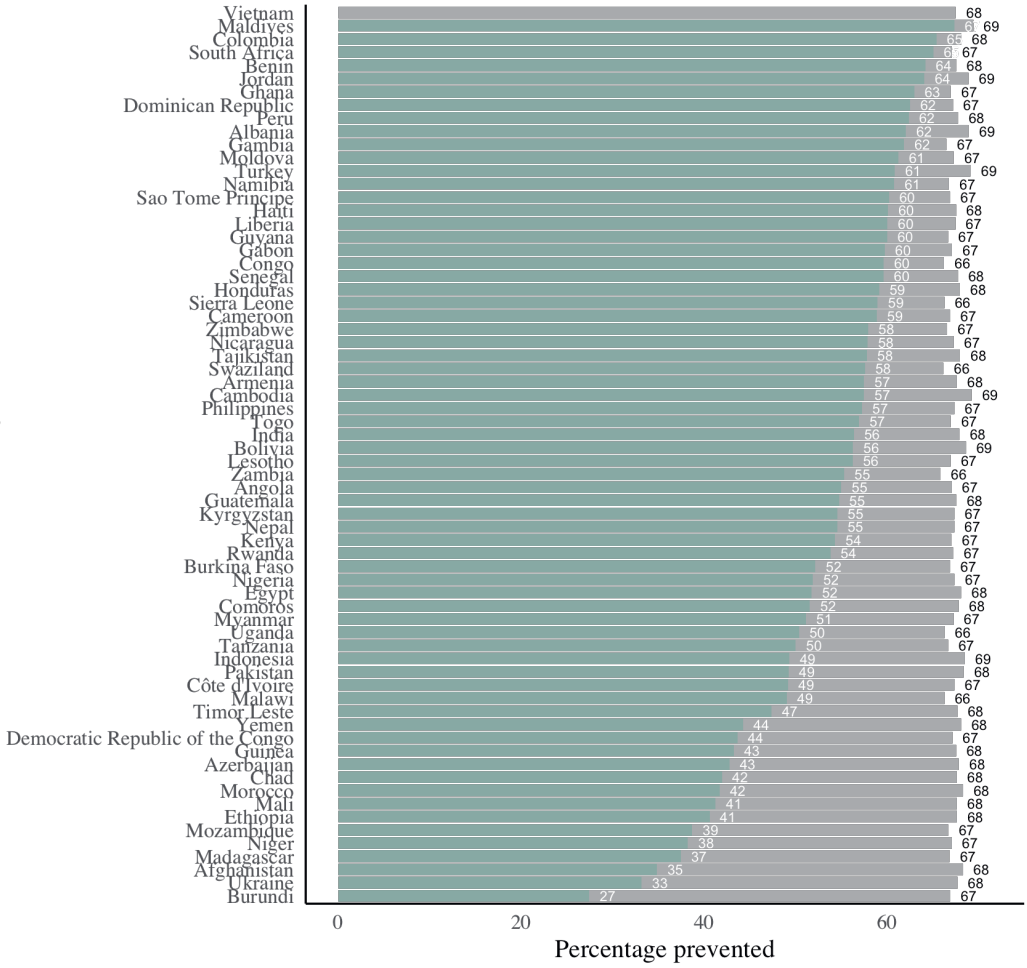



**Figure 4B**: Country-specific estimates for impact of maternal vaccination on RSV-B related in-hospital mortality, assuming full vaccine coverage (grey) and a realistic vaccine coverage (green).

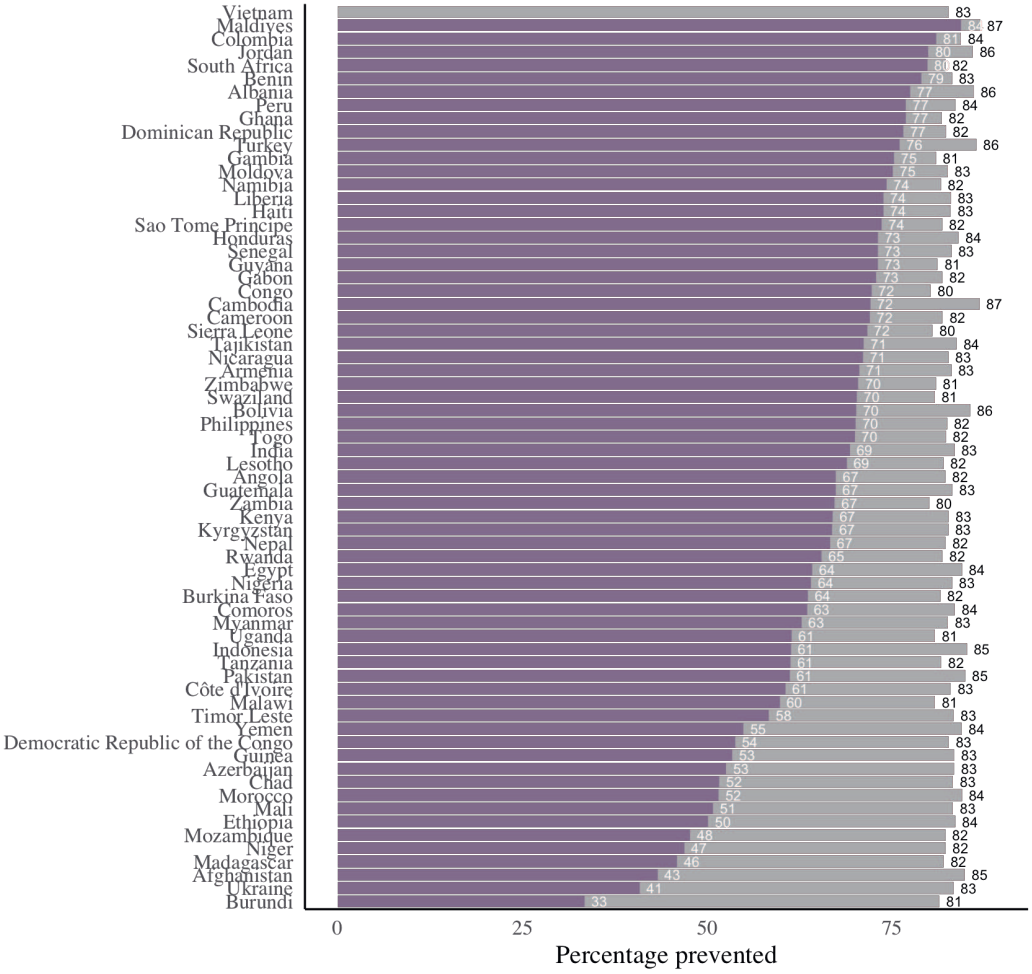



**Figure 5A**: Country-specific estimates for impact of maternal vaccination on RSV-A related out-of-hospital mortality, assuming full vaccine coverage (grey) and a realistic vaccine coverage (purple).

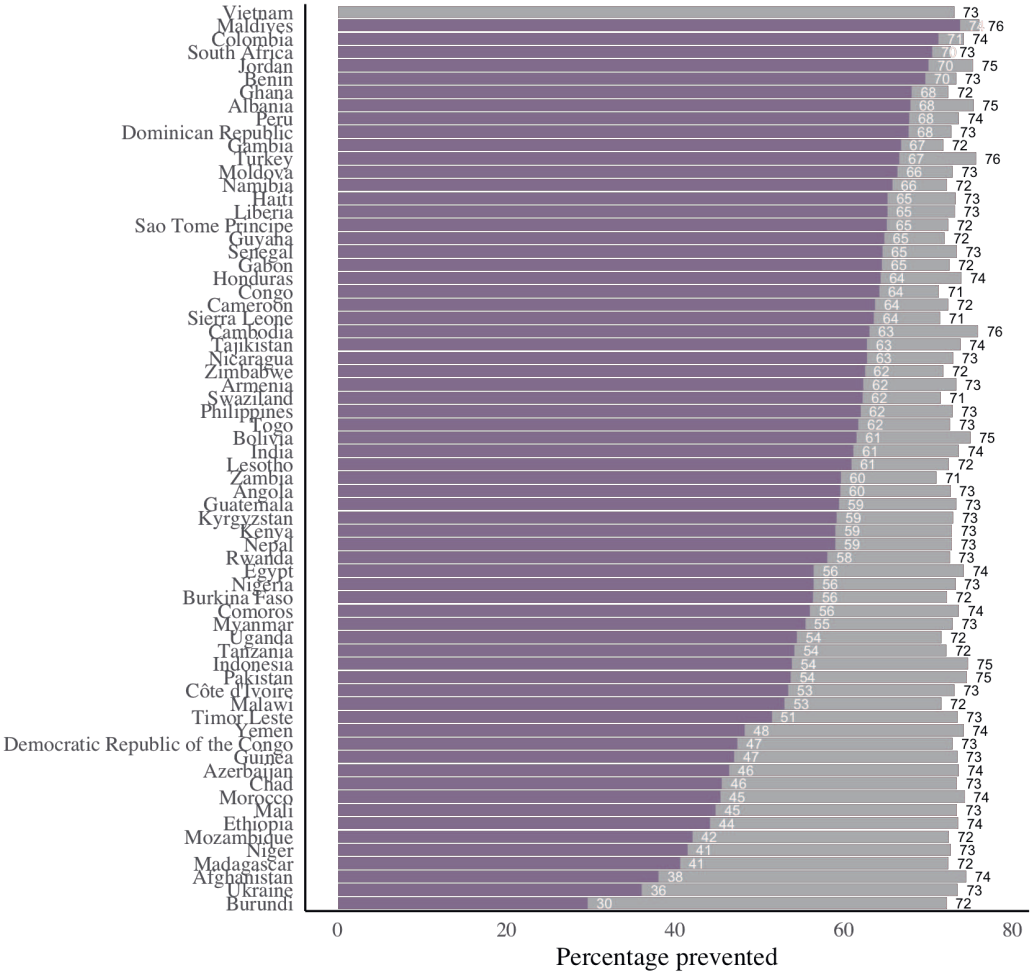



**Figure 5B**: Country-specific estimates for impact of maternal vaccination on RSV-B related out-of-hospital mortality, assuming full vaccine coverage (grey) and a realistic vaccine coverage (purple).

## DISCUSSION

We have refined and extended a previously published mathematical model [10] to estimate vaccine efficacy, defined as the percentage of averted RSV-related mortality cases before 6 months. The model was calibrated using the phase 1/2 RSV prefusion F (RSVpreF) vaccine trial [11]. It was applied to simulated data sets based on the observed distributions of gestational age at time of birth and age at time of death in the RSV GOLD database. According to our model, the probability that RSV mortality before 6 months of age is averted by the RSVpreF vaccine depends strongly on gestational age at time of birth. Assuming that early vaccination is deemed entirely safe concerning preterm birth, the most favorable timing for administering the RSVpreF vaccine would be early in pregnancy. From an implementation perspective, it may be crucial to consider the timing of maternal vaccines' administration in a way that balances optimal antibody response timing while also ensuring maximum vaccine coverage or maximum coverage of other concurrently delivered interventions.

The model predicted that given a probable vaccination coverage, the RSVpreF Vaccine, that recently received approval from the EMA, could decrease the yearly number of RSV-related cases in 51 GAVI-eligible countries in the first half year of life by 15000 to 17000. This corresponds to a weighted average of 55% to 63% of all RSV-related mortality cases in the first year. Future research should translate these findings into cost-effectiveness analyses to guide policy makers with prioritizing interventions that have the highest impact and best value for money.

In our previous work [10], we estimated a mortality reduction range of 29-48% in the global mortality cohort based on the Novavax vaccine, which is notably lower compared to our current estimates. However, it should be noted that the Novavax vaccine, as evaluated in the NCT02624947 phase 3 trial [17], did not meet its primary endpoint of medically significant RSV lower respiratory tract infection (LRTI) and clinical trials were not pursued. To the best of our knowledge, this is the first mathematical modelling study predicting the potential impact of the promising RSVpreF Vaccine in LMICs. Our estimates are in line with data from the phase 2b and phase 3 RSV PreF trial [18,12], where data on infant neutralizing titers suggest that the maternal vaccine has the potential to protect infants from RSV infection well into their first 6 months of life [18,12]. The phase 3 RSV PreF trial efficacy analysis showed efficacy of 76.9% for severe medically attended RSV-associated lower respiratory tract illness during the first 180 days in LMICs [27]. Considering that RSV-B was predominant during the trial, our estimates for potential impact in LMICs for a vaccination window between 24 and 36 weeks are in line with the phase 3 trial efficacy estimates. The vaccine efficacy estimates for RSV-A specifically in the phase 3 trial are notably lower compared to the estimates from our model, however these estimates are surrounded

by large confidence intervals due to a limited number of RSV-A cases in the phase 3 trial and might therefore be less reliable.

In our model we fitted the antibody transfer function on observed cord blood to maternal ratio IgG levels as described previously (ref [10]), as this is the best available data to date. However, besides gestational age, the rate of antibody transfer also depends on the type and subclass of the antibody [28,29,30,31] as well as vaccine formulation and population of interest[17,18]. Importantly, our predicted antibody transfer ratios, and subsequent fetal antibody levels at time of birth, are lower than observed in the RSVpreF phase 2b trial (where transfer ratios ranged from 1.41 to 2.10)[18], leading to more conservative vaccine impact estimates. In order to improve the prediction of the impact of maternal vaccination, especially for preterm infants, and to improve the prediction of the optimal timing of RSV immunization in pregnancy it is essential to have more granular data evaluating the antibody transfer by week of gestational age [32,33].



Another important assumption in our model is the choice for the protection threshold. We assumed that palivizumab serum titers, or RSV neutralizing titers corresponding to palivizumab serum titers, equal or above 100 µg/*ml* could avert (or at least postpone) RSV-related mortality. Although this threshold has been the best established correlation with protection to date [34,35,11], studies that have investigated other immunological correlates of protections against severe RSV disease have yielded inconsistent findings [36]. As we use the protection threshold to predict the number of days protected, a lower or higher threshold would lead to a under- or overestimation of the number of days protected and therefore different impact estimates. Having a better knowledge on correlates of protection is essential to improve the prediction of the impact of maternal vaccination. Lastly, we assumed that the neonatal antibody half-life of the maternally derived RSV specific antibodies is independent on time, prematurity, weight, or other biological factors. There are some indications that the antibody half-life might be shorter in preterms and in the first 4 months of life [37].

A strength of our study is the use of characteristics of a maternal RSV vaccine currently in development [23] and data simulation based on real data from a global mortality registry [22]. Our model has multiple applications. Firstly, it can be applied to estimate the duration of protection provided by maternal vaccination on case-level, based on gestational age at time of vaccination and gestational age at time of birth. As little research has been carried out on the efficacy of maternal vaccines in prematurely born infants [8], our model can contribute to inform at which gestational age we can assume that infants are protected when born prematurely and contribute to bridge vaccine efficacy to this population. Secondly, our model can be applied to estimate vaccine efficacy on a group or population-level to bridge vaccine efficacy to populations not included in initial efficacy trial(s). Thirdly, our model can help to

inform optimal timing of RSV immunization in pregnancy. Lastly, after reparameterization, our model can be applied to other vaccines that can be recommended during pregnancy such as tetanus, diphtheria, pertussis, and influenza, as maternal neutralizing antibodies against these pathogens are also transferred to the fetus during pregnancy [38].

One limitation of our study is the reporting bias, quality, and completeness of the RSV GOLD database, which have been described elsewhere [20,21,22]. In our study, gestational age was often missing and was considered unreliable in the LMIC subset in our database. We simulated data based on the most reliable gestational age at time of birth distribution across the full RSV database, including high income countries. Furthermore, for the data simulation we assumed there is no relationship between gestational age at time of birth and age at time of death. Although we did not observe a significant correlation in the RSV GOLD subset, it is not unlikely that a correlation exists, which would lead to bias in our estimates. In addition, for simplicity, and due to a lack of data, we did not incorporate country-specific distributions for age at time of death and gestational age. Our model does not account for comorbidity or other biological factors that may impair antibody transfer, such as low birthweight, maternal HIV infection, and placental malaria [32], despite their high prevalence in LMICs. We did not incorporate breastfeeding into our model, as research on orally administered antibodies has yielded no significant increase in serum titers of intact antibodies [39]. It should also be noted that the estimates for timing of ANC visits in LMICs by gestational age that we used in our model, were based on data published between 2001 and 2018 [24] and might therefore be dated. Furthermore, there's a large uncertainty around the used service availability and acceptance proxy and mortality burden estimates used to make country-specific predictions. For the estimated number of cases averted we assumed a 1:3 in-hospital to out-of-hospital mortality ratio for a crude estimate. However, in a different analysis the estimate of RSV mortality rate has been estimated to be 7- to 10-fold higher out-of-hospital compared with the in-hospital death [40]. Assuming a 1:10 in-hospital to out-of-hospital mortality ratio would change our results to between 47879 and 41956 in-hospital mortality cases total (estimate based on vaccine efficacy for RSV-A and RSV-B respectively) averted yearly in those 51 Gavi-eligible countries.

## CONCLUSION

We predicted that maternal vaccination against RSV could substantially decrease the number of RSV related in-hospital and out-of-hospital deaths before 6 months of age in LMICs. Our model can be used to estimate duration of protection provided by maternal vaccination on case-level and to help inform policy makers on optimal timing of maternal vaccination during pregnancy. It can give an indication of the population-level effectiveness of new interventions prior to introducing a preventive strategy, which is critical for governments and large funding organizations.

## NOTES

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*Authors' contributions*

JW and JD conceptualized the study. JD and JB advised on methods and supervised analyses. JW did the analyses and modeling analyses. LB assisted with data collection. JW and JD wrote the first draft of the article, with further contributions from JB and LB. All authors interpreted data and approved the final version of the article. All authors had access to the data and had responsibility for the decision to submit for publication.

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# Chapter 5

## Disagreement FDA and EMA on RSV Maternal Vaccination: Possible Consequence for Global Mortality

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PIDJ 2024 Jan 1;43(1):e1-2

## ABSTRACT

The European Medicines Agency and the U.S. Food & Drug Administration (FDA) have recently approved a maternal vaccine for respiratory syncytial virus. The FDA limits vaccination to later in pregnancy. Mathematical modeling demonstrates that this vaccination window may reduce the global mortality impact of the vaccine by 12%. Policymakers should carefully consider vaccine risks and benefits to safeguard vulnerable infants effectively.

## Commentary

Both the European Medicines Agency (EMA) and the U.S. Food & Drug Administration (FDA) recently approved a novel maternal vaccine against respiratory syncytial virus (RSV) infection. Remarkably, they recommended differently on the timing of vaccination [1,2]. While the EMA recommends vaccination at 24-36 weeks of pregnancy, which is in line with the timing of administration during the registration trial, the FDA recommends vaccination at 32-36 weeks of pregnancy. In this Perspective, we discuss the consequences on global mortality if policymakers would follow the FDA's recommendation of limiting maternal vaccination to late pregnancy.

A recent registration trial with the registered subunit RSV vaccine, marketed as Abrysvo, showed the vaccine's effectiveness in reducing severe RSV disease within the first six months of life (NCT04424316) [3]. The different recommendations of the EMA and FDA with respect to the moment of vaccine administration is due to different interpretations of safety data of this trial. The EMA concluded that there are no major safety concerns and therefore decided that the benefits of vaccination are greater than its risks, and that the vaccine can be administered relatively early during pregnancy. The FDA, on the other hand, stressed a small (albeit non-significant) increase in premature birthrates in the vaccine-receiving cohort in this trial. Abrysvo is not the first maternal RSV vaccine where concerns were raised about a possible increase in preterm births. Previously, GSK trials of a similar product were halted due to a significant rise in preterm births, which was associated with neonatal mortality [4]. Because there is currently not enough evidence for or against a causal relationship between the vaccine and an increased risk of premature birth, as a precaution, the FDA restricted vaccination to after the 32nd week of pregnancy.



The next crucial goal is to secure vaccine licensure and access in regions that are disproportionately badly affected by RSV, particularly in low- and middle-income countries (LMICs). The vast majority (97%) of RSV-attributable deaths occur in LMICs, and almost half of them in the first 6 months of life [5]. While in high-income countries there is the alternative option of monoclonal antibodies administration to protect infants early in life, it is unlikely to be feasible in LMICs. Before formulating vaccination guidelines for LMICs, it is imperative to carefully weigh the risks and benefits of different vaccine administration timings. Delaying vaccination has a clear drawback: lower vaccine coverage among prematurely born infants, who are already at higher risk. On the other hand, antibody transfer across the placenta is less efficient in the early stages of pregnancy, potentially reducing the benefits of early vaccination. Weighing these two opposing factors is crucial to determine the most effective moment of vaccination in LMICs.

We recently used a mathematical model to predict the potential impact of maternal RSV vaccination on preventing RSV-related deaths dependent on the moment of vaccination[6]. The model describes how maternal antibodies rise and fall after vaccination, to what extent these antibodies are transported via the placenta to the unborn child, the resulting antibody levels at time of birth of the infant, and the predicted period after birth during which antibody levels are sufficiently high to protect the infant from severe RSV infection. The model was parameterized on the clinical characteristics of the Abrysvo vaccine and on mortality data from LMICs.

Based on this model, we concluded that, if early vaccination is safe concerning preterm birth, the most favorable timing for vaccine's administration would be between 24 and 27 weeks of pregnancy. The FDA restricts vaccination to after the 32nd week of pregnancy and since it takes approximately 2 weeks after vaccination before an effective immune response is mounted, children born before the 34th week of pregnancy will not benefit from the vaccine according to the FDA guideline. Under the EMA guidelines, however, infants born before 34 weeks of pregnancy could still be protected against RSV for up to 142 days by vaccinating their mothers relatively early during pregnancy.

**Figure 1** compares the maximal impact of maternal vaccination at 24 weeks (EMA-guidelines) and 32 weeks of pregnancy (FDA-guidelines) on the fraction of preventable RSV deaths. Delaying vaccination until the 32nd week of pregnancy results in a lower fraction of children protected at birth, as infants born before the 32nd week will be missed in the vaccination effort and infants born between 32 and 34 weeks will not yet benefit from the vaccine. On the other hand, delaying vaccination until later in pregnancy will protect the infants of vaccinated mothers for a slightly longer period after birth, as maternal antibodies gradually decline from two weeks post-vaccination onward. The optimal trade-off between vaccinating earlier or later in pregnancy is thus influenced by the proportion of children born before the 34th week of pregnancy, which was 19% based on the RSV mortality data [6]. As children who are born prematurely are more vulnerable for severe RSV infection and RSV-related mortality, this proportion is higher than in the general population. Importantly, it is exactly this group of children who are born prematurely who need to be protected by maternal vaccination.

To estimate the number of lives potentially saved by vaccination, the likelihood of RSV-related death at different ages needs to be taken into account. Based on age-at-death data from mortality data in LMICs, we calculated that vaccination at 24 and 32 weeks of pregnancy could prevent up to 94% and 82% of all RSV mortality cases, respectively. Considering the estimated total number of RSV mortality cases under 6 months old in

LMICs [4], maternal RSV vaccination can make a difference of 3,300 deaths per year. However, an impact trial is necessary to fully understand the overall impact of maternal vaccination in LMICs. To protect vulnerable infants as much as possible, we urge policymakers to strike a careful balance by not only considering safety issues regarding vaccine-associated risks that cannot yet be ruled out, but also the potential reduction in the number of deaths achieved by vaccinating mothers relatively early in pregnancy.

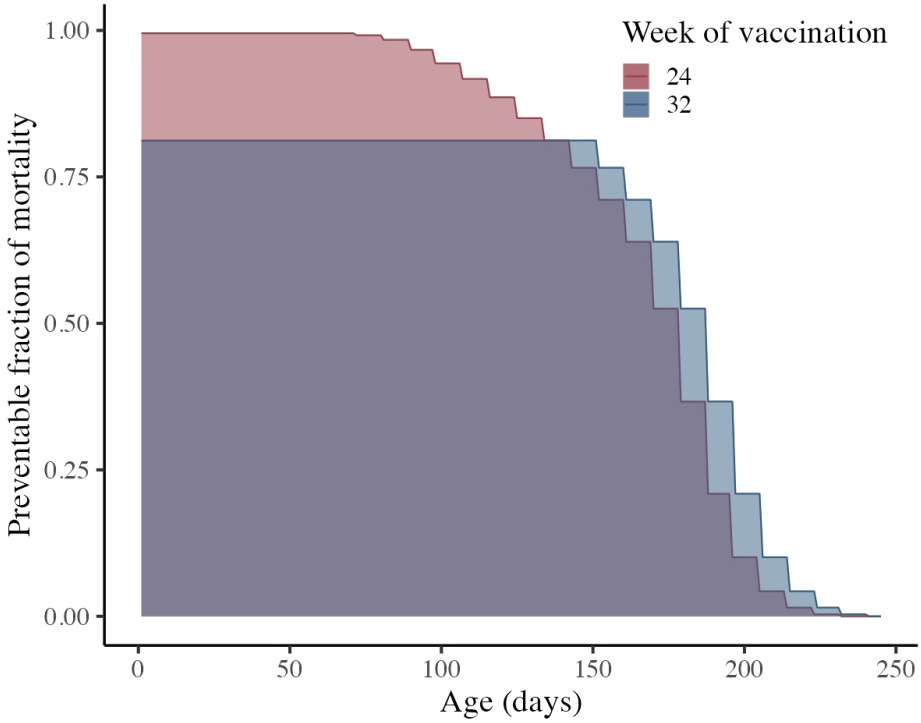



**Figure 1**: The plot illustrates the maximal fraction of preventable RSV-related mortality cases across various infant age groups based on the RSV mortality data, assuming maternal vaccination occurs in either the 24th week (in red) or the 32nd week of pregnancy (in blue). In essence, it provides insight into the percentage of infants born with sufficient immunity to RSV acquired through maternal vaccination and tracks the gradual decline of this vaccine-induced immunity over time.

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# Part III

## Antibody specific kinetics



"It is easy to make things hard, but hard to make things easy"
- Rutger Bregman

# Chapter 6

## Low Cord Blood Titers Drive Prolonged Maternal RSV Antibody Half-Lives in Preterm Infants

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Submitted

## ABSTRACT

**Introduction**:

Preterm infants are at higher risk of severe RSV infection, partially because of reduced placental transfer of maternal antibodies (Abs). Increased risk of severe infection persists in the first year of life. This study evaluates the postnatal kinetics of RSV neutralizing antibodies in preterm and full-term infants born to unvaccinated mothers.

**Methods**:

We enrolled infants (24–42 weeks GA) born in hospital from RSV-unvaccinated mothers. Serum was collected at birth (cord blood), 1–2 weeks, and 4–8 weeks. Linear mixed-effects models were used to estimate half-lives of RSV-neutralizing Abs in relation to GA and cordblood titer. Analyses were repeated for total serum IgG1 to confirm the robustness of our findings.

**Results**:

Of 169 infants, 95 were born preterm (<37 weeks GA, including 53 neonates <32 weeks GA). Premature birth was related to prolonged half-life of RSV-neutralizing Abs (~79 days very preterm to ~30 days in term infants). The inverse association between gestational age and antibody half-life was almost completely driven by an inverse association between cord blood Ab levels and Ab half-life. A doubling in cord blood titers led to a reduction in half-life of ~20% for RSV neutralizing Abs and ~32% for IgG1.

**Conclusions**:

Postnatal antibody half-life depends on starting levels: higher titers decline faster. If our observation would be replicated for vaccine-induced Abs, the difference in vaccine protection between preterm and term infants may be smaller than anticipated.

## INTRODUCTION

Respiratory syncytial virus (RSV) is a major global cause of lower respiratory tract infections and a leading contributor to hospitalizations and mortality among children under five years of age [1,2]. Infants under two years, particularly those younger than six months and those born preterm, are at greatest risk of severe RSV disease [3]. The recent approval and rollout of maternal RSV vaccines in several countries represent an important advance in reducing the early-life burden of RSV [4,5]. Maternal immunization aims to confer passive protection through transplacental antibody transfer. However, trial eligibility criteria in Phase 2b [6] and Phase 3 [7] studies limited enrollment of preterm infants, leaving a critical evidence gap on vaccine effectiveness in this high-risk group.

Passive protection is determined by three factors: the maternal antibody response, the efficiency of placental transfer, and the antibody half-life. Maternal/cord transfer ratios increase later in gestation [8–10], as a result preterm infants are born with lower maternal antibody levels. [8–10] Importantly, antibody concentrations at birth itself may also influence antibody half-life: earlier studies from the 1970s suggested that IgG decay is proportional to antibody concentration, a phenomenon known as the concentration–catabolism effect, in which high concentrations accelerate catabolism while low concentrations slow it [11,12]. This effect may be explained by saturation of the neonatal FC receptor (FcRn), which normally protects IgG from degradation. At higher antibody concentrations, FcRn may become saturated, limiting its binding capacity and resulting in more rapid catabolism [13,14]. First proposed by Brambell in 1964 [11,12], this mechanism has been supported by experimental studies [14,15] and observations for measles [16], rubella [17]. More recent, indirect support can be inferred from studies of maternal pertussis vaccination [18] and maternal RSV antibody levels [19], both showing that higher antibody concentrations at delivery or in cord blood were associated with shorter infant antibody half-lives.

The implications of the concentration-catabolism effect for antibody decay in preterm infants specifically have not, to our knowledge, been explored. This study evaluates the postnatal kinetics of RSV-A–neutralizing antibodies in preterm and full-term infants born to unvaccinated mothers. We quantified antibody half-lives across gestational ages, examined their relationship with antibody levels at birth, and validated findings using total IgG1 concentrations. Finally, we modeled a hypothetical maternal vaccination scenario to examine the clinical implications of differences in antibody half-life.

## MATERIALS AND METHODS

*Study Design*

This substudy was part of an international seroepidemiology program initiated by Pfizer to assess transplacental transfer of naturally acquired RSV-neutralizing antibodies.[20] The present follow-up was conducted among participants enrolled at the University Medical Centre Utrecht/Wilhelmina Children's Hospital, the Netherlands; between April 2022–April 2024). These mother–infant pairs were recruited at delivery, and for the current analysis only infant samples and data were used. Infants were eligible if born to mothers ≥18 years of age who were hospitalized for imminent delivery from 24 weeks' gestation onwards.

We recruited infants into three gestational age groups with a 1:1:1 ratio: very preterm ($24^{0/7}$–$31^{6/7}$ weeks), moderately preterm ($32^{0/7}$–$36^{6/7}$ weeks), and term (≥ 37 weeks). These categories were defined to align with the study objectives and differ from the World Health Organization (WHO) definitions (extremely preterm: <28 weeks; very preterm: 28–<32 weeks; moderate-to-late preterm: 32–<37 weeks). To maintain balance between groups, enrollment of infants ≥37 weeks' gestation was discontinued once 70 term births had been included.

Infants were excluded if specimen collection failed within 24 hours after birth. For preterm infants (<37 weeks), blood samples were collected at birth (cord blood, T0), at 1–2 weeks of age (T1), and at 4–8 weeks (T2). For term infants (≥37 weeks), samples were collected at birth and at T2 (Supplementary Table 1). To ensure unbiased estimation of postnatal antibody half-life, follow-up serum samples were excluded if titers were likely influenced by external factors. Specifically, samples were excluded if: the infant had a confirmed RSV infection, defined as reported symptoms of acute respiratory infection (ARI) in combination with a ≥2-fold increase in antibody titers between visits, the infant received plasma or intravenous immunoglobulin (IVIG) transfusions with concurrent outlier titers or if palivizumab was administered during the follow-up period.

Participants were eligible for IgG1 analysis if sufficient serum remained after neutralizing antibody assays, and only if ≥2 samples were available. For this analysis, follow-up samples were excluded from three infants who had received plasma or IVIG transfusions in combination with outlier IgG1 concentrations

*Variables and specimen testing*

To assess the half-life of maternally derived RSV-specific antibodies in neonates across gestational ages, we collected repeated serum samples and relevant medical data from the infant. Infant medical data included birth weight, gestational age at birth, comorbidities (e.g., congenital heart disease), palivizumab administration, blood product transfusions (with type), and the number and estimated volume of blood draws, as part of routine clinical sampling, during hospitalization. At follow-up, we collected data on blood transfusions, reported respiratory symptoms, RSV immunization, and any laboratory-confirmed RSV infections.

RSV A–specific neutralizing antibody titers were measured using a fluorescence-based micro-neutralization assay. Total serum IgG1 concentrations were measured using the Optilite® IgG1 assay (The Binding Site, Birmingham, UK). Detailed laboratory procedures are provided in the Supplementary Methods S1.

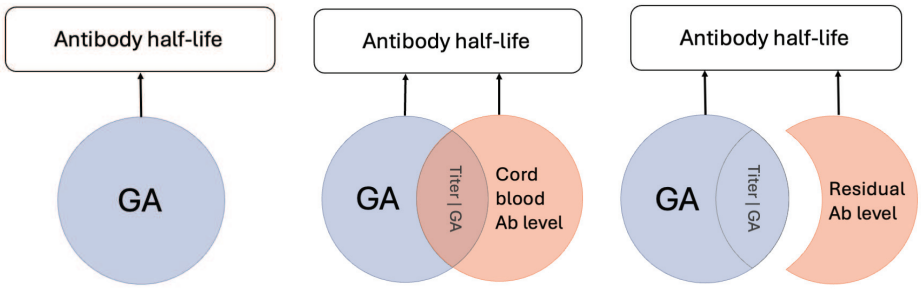
*Statistical analysis*

For each infant, the cord blood RSV-neutralizing antibody (RSV NAb) titer was defined as the cord blood measurement at birth or, if unavailable, the first follow-up sample obtained within two weeks.

We modeled longitudinal antibody trajectories using linear mixed-effects models, with time (days since first sample) as the primary predictor and random slopes for time to account for between-infant differences in decay rates. Gestational age (centered at 40 weeks) was included as a fixed effect interacting with time to assess whether maturation at birth influenced the rate of antibody loss.

Because gestational age and cord blood antibody level are strongly correlated, we applied three complementary modeling approaches to separate their effects on antibody half-life (**Figure 1**). The first model (M1) estimated the unadjusted association between gestational age and half-life, and the second model (M2) included both gestational age and cord blood antibody level to disentangle their independent contributions. In the third model (M3), we replaced cord blood antibody level by a residualized titer, defined as the difference between an infant's observed and expected antibody level for their gestational age. This approach retains the complete maturational effect of gestational age, consistent with the first model. It then evaluates whether residual variation in antibody titer, beyond that attributable to gestational age, is associated with antibody half-life. Antibody half-lives were derived based on regression coefficients from this model. Specifically, the half-life was estimated as the negative inverse of the time coefficient (including all time–covariate interaction terms)[18]. Finally, we extended the third model to include breastfeeding

status and the number of blood draws during hospitalization to explore potential postnatal influences on antibody half-life. Full model specifications and derivations are provided in the Supplemental Materials (S1: Supplementary Methods).




A B C

**Figure 1.** Conceptual illustration of the three modeling approaches used to assess the relationship between gestational age (GA), cord blood antibody (Ab) level, and antibody halflife. **A**) M1: tests whether GA alone predicts antibody half-life, reflecting the overall, unadjusted relationship between maturational stage and antibody half-life. **B**) M2: examines independent effects of GA and cord blood antibody level. These predictors are strongly correlated, their shared variance is denoted as "Titer | GA". **C**) M3: replaces cord blood titer by its residual after adjusting for GA. As in M1, this model captures the full maturational effect (including Titer | GA), while the residualized titer reflects infant-specific deviations independent of GA (e.g. lower or higher levels than expected based GA alone).

To assess potential impact of our findings if they are applicable to vaccine-induced antibodies, we performed an additional modeling study (S2. Supplementary Analysis). To validate if the observed results are specific to RSV-A antibody or reflect broader antibody kinetics, we repeated the same analyses using total IgG1 concentration, as RSV-A neutralizing antibodies are predominantly of the IgG1 subclass [21] and the neonatal production of IgG starts after about 6 months of age [22].

We performed several sensitivity analyses to assess robustness of the findings: repeating models stratified by term versus preterm infants, excluding those who received blood products or showed increasing postnatal titers, and restricting the RSV NAb analysis to infants with IgG1 measurements. Detailed model specifications and results can be found in the Supplemental Materials (S3: Sensitivity Analysis).

## RESULTS

*Study cohort*

We enrolled 169 infants at birth (**Table 1**), including 95 (56%) preterm infants, and followed them for up to 8 weeks. We excluded follow-up samples from 10 infants: one with confirmed RSV infection, three with ARI symptoms and ≥2-fold antibody rises, three following blood transfusion, and three from infants receiving palivizumab during follow-up. After these exclusions, 388 and 239 samples remained available for RSV neutralization and IgG1 measurement (**Table 1**, **Figure 1**, Supplementary Table 3).

**Table 1.** Number of available samples for RSV-A neutralization and clinical characteristics of infants by gestational age group.

| Category | N | $N_{T0}$ (birth) | $N_{T1}$ (1-2 wks) | $N_{T2}$ (4-8 wks) | Gestational age Median (IQR) | Comor-bidity (≥1) n (%) | Breast-feeding % (n) | Standard blood draws Median (IQR) |
|---|---|---|---|---|---|---|---|---|
| Very preterm 24–32 weeks | 53 | 51 | 45 | 38 | 31 (30–32) | 48 (90.6) | 62 (33) | 24 (18-52) |
| Preterm 33–36 weeks | 42 | 37 | 34 | 35 | 35 (34–36) | 27 (64.3) | 57 (24) | 11 (4-17) |
| Full-term 37–41 weeks | 74 | 74 | – | 74 | 39 (38–40) | 21 (28.4) | 72 (53) | 2 (0-5) |

IQR: InterQuartile Range, wks: weeks
$N_{t0}$, $N_{t1}$, and $N_{t2}$ indicate the number of infants with samples available for RSV-A neutralization analysis at birth (T0), 1–2 weeks (T1), and 4–8 weeks (T2).

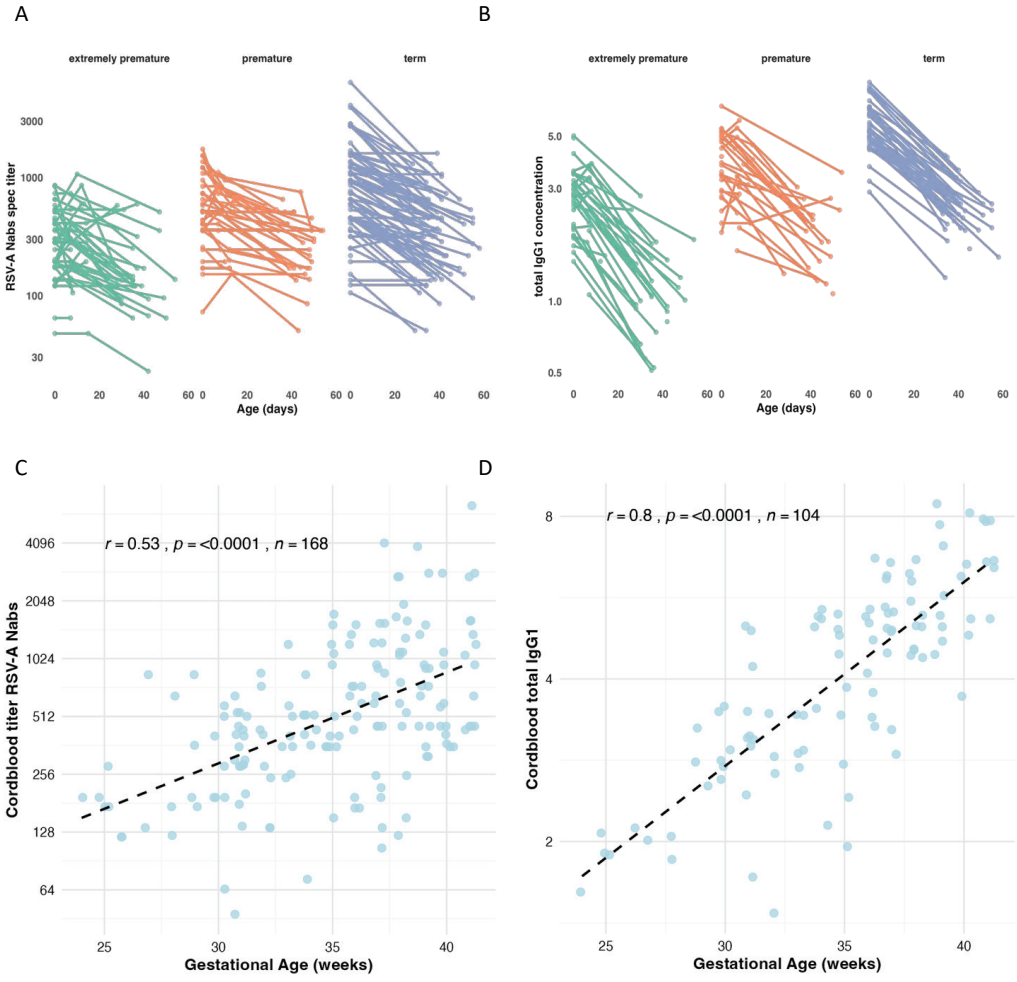



**Figure 2.** Individual trajectories of RSV and IgG1 over time and correlation between gestational age and antibody levels.

**A**) Individual trajectories of RSV neutralizing titers over time.

**B**) Individual trajectories of IgG1 concentration over time.

**C**) Correlation between infant gestational age and cord blood RSV-neutralizing titers (Pearson's r = 0.53, p < 0.0001, n = 168).

**D**) Correlation between infant gestational age and cord blood total IgG1 concentration (Pearson's r = 0.8, p < 0.0001, n = 104).

*Half-life estimates*

RSV titers

In line with previous studies [8,9], higher gestational age was associated with higher cord blood antibody titers (β = 0.160 per week increase, $p < 0.001$; $R^2 = 0.28$, $p < 0.001$; **Figure 2B**), confirming that premature infants are born with lower antibodies. We then tested the effect of gestational age (GA) on the estimated half-life of RSV antibodies (M1). We found that lower GA was associated with a longer half-life (**Table 2**). The effect of GA was no longer significant after including cord blood titers in our model (M2, **Table 2**), suggesting that the observed association between GA and half-life was driven by the inverse association between cord blood antibody levels and half-life. We then fitted a third model (M3), in which the association between gestational age and half-life was held constant, to assess whether residual variation in cord blood titer (independent of gestational age) explained additional variability. In this analysis, both higher gestational age and higher residual titers were associated with significantly shorter half-lives (**Figure 3B**, **Table 2**). For example, at 40 weeks' gestation, doubling the cord blood titer reduced the estimated half-life from approximately 32 to 26 days (≈20% shorter). For the detailed model output, see the Supplementary Materials (Supplementary Table 4).

**Table 2.** Effects of Variables on the Half-life in the Respective Model for the Respective Antibody.

| Variable | M1 (GA) | | M2 (GA + CB Ab titer) | | M3 (GA + Residual CB Ab titer) | | M3 + Breastfeeding | | M3 + Number of blood draws* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **RSV** | **IgG1** | **RSV** | **IgG1** | **RSV** | **IgG1** | **RSV** | **IgG1** | **RSV** | **IgG1** |
| GA | – | NS | NS | + | – | + | – | + | – | NS |
| CB Ab titer | | | – | – | | | | | | |
| Residual CB Ab titer | | | | | – | – | – | – | – | – |
| Breastfeeding | | | | | | | NS | NS | | |
| Number of blood draws* | | | | | | | | | – | – |

Abbreviations: CB, cord blood; GA, gestational age; Ab, Antibodies; M, model; NS, not significant. + indicates a significant increase in the half-life, ie, decreasing the speed of decay, while – indicates a significant decrease in the half-life, ie, increasing the speed of decay. The effect refers to an increase in the respective variable. For the detailed model output, see the Supplementary Material. *Number of blood draws during follow-up as part of routine clinical sampling.

IgG1 concentration

We applied the same modeling strategy used for RSV antibodies to total IgG1 concentrations. Later gestational age was again associated with higher cord blood antibody levels (β = 0.113 $\log_2$-units per week, $p < 0.001$; $R^2 = 0.64$, $p < 0.001$; **Figure 2D**). For IgG1, gestational age showed the opposite relationship with half-life compared with RSV antibodies: preterm infants had shorter IgG1 half-lives (**Table 2**, Supplementary Table 4). Nevertheless, the effects of cord blood and residual titers were directionally consistent with the RSV analysis. At 40 weeks' gestation, doubling the cord blood IgG1 concentration shortened the half-life from ~41 to ~28 days (≈32% shorter).

*Breastfeeding and number of blood draws*

We next examined whether postnatal factors influenced antibody half-life. We did not find evidence that breastfeeding was associated with antibody half-life of RSV neutralizing Abs or IgG1 (Supplementary Table 4). A higher number of blood draws (as part of routine clinical sampling) was significantly associated with faster antibody decay (Supplementary Table 4), corresponding to a ~0.3 day shorter half-life per additional sample.

*Sensitivity analyses*

Across all sensitivity analyses, effect estimates were consistent with the main models (Supplement S3). Higher (residual) cord titers were consistently associated with shorter half-lives, while higher gestational age was linked to shorter half-lives for RSV-A but longer half-lives for IgG1.

*Impact vaccine*

To assess potential impact of our findings, if they are applicable to vaccine-induced antibodies, we performed a concise modeling study (Supplement S2). These simulations showed that the difference in days protected after birth (protection gap) remains between term and preterm infants (**Supplemental Figure 3**). However, this gap reduces as cord antibody titers rise through vaccination, for instance, at a 2-fold rise in antibodies the protection gap spanned 73 days, whereas at a 10-fold rise it fell to only 28 days. Analyses that ignore the dependence of antibody half-life on baseline titer overestimate the protection gap between preterm and term infants, particularly at higher antibody concentrations following maternal immunization.

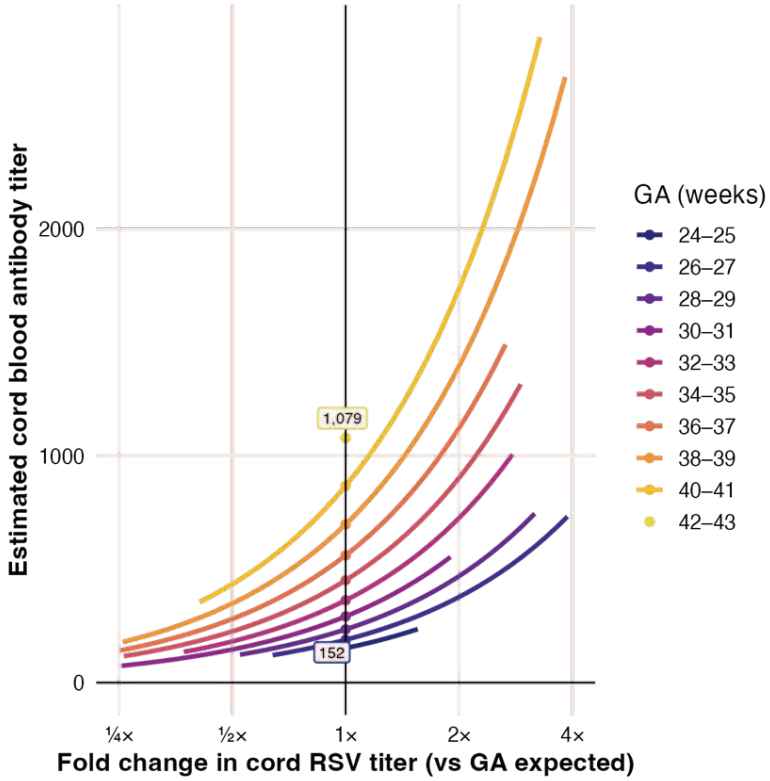





**Figure 3A.** Impact of gestational age (GA) and infant-specific deviations in cord antibody level on cord antibody titer (Model M3). Cord blood RSV neutralizing antibody titers, expressed as fold change relative to the average level expected for each GA. Color shading indicates gestational age, illustrating that average titers increase with advancing maturity.

Note: Lines are shown only for values observed in the study. Fold change represents how much higher or lower the antibody level is compared with the average for that gestational age. A fold change of 1 corresponds to the average expected level (shown at the y intercept). Values >1 indicate higher-than-expected levels, while values <1 indicate lower-than-expected levels. For example, a fold change of 2 means the antibody level is twice as high as expected, whereas a fold change of 0.5 means it is half as high.

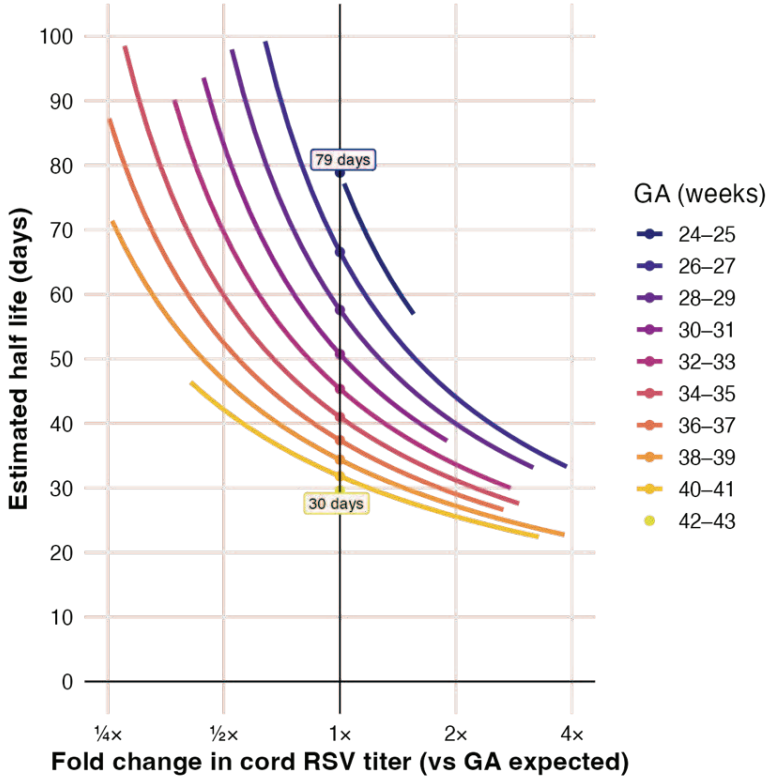



**Figure 3B.** Impact of gestational age (GA) and infant-specific deviations in cord antibody level on antibody half-life (Model M3). Estimated antibody half-life (in days) in relation to GA and individual deviations in cord antibody level. This figure shows how both overall maturity (including higher titers at older GA) and infant-specific differences in antibody levels are related to antibody half-life.

## DISCUSSION

This study characterizes the decline of RSV-neutralizing antibodies (RSV nAbs) after birth in infants born between 24 and 42 weeks of gestation to RSV-unvaccinated mothers. We found that antibody half-life is influenced by the cord blood antibody concentration at birth. This suggests that although preterm infants start with lower antibody levels, the vaccine-derived protection gap between preterm and term infants is smaller than would be expected if concentration-dependent half-life is ignored.

A plausible biological explanation for the observed relationship between antibody levels and half-life is the concentration–catabolism effect [14], in which high IgG levels decrease faster due to saturation of FcRn-mediated recycling. The dependence on starting titers, together with population-level differences in baseline antibody levels, may underlie part of the large variability in half-life estimates of RSV nAbs [13,19,23–25] in literature.

When considering the effect of gestational age on antibody half-life, we found conflicting results depending on the model and antibody. Differences in early-life exposure, with full-term infants generally discharged earlier and thus encountering more environmental antigens, might also influence antibody kinetics, yet even under these conditions RSV nAb half-lives remained shorter in full-terms. The discrepancy between our RSV and IgG1 results, and across published studies reporting both significantly longer [18,26] and shorter [27–29] half-lives of maternal antibodies in preterm infants, suggests that gestational age effects on half-life are complex. Prematurity itself might shorten half-life, for example, through illness-related metabolic demands [10,28,29], but this may be outweighed by the indirect effect of lower cord blood titers in preterms, which are associated with longer half-lives. We hypothesize that these opposing forces could help explain why half-life differences between preterm and term infants vary in our study and across studies. We also found that a higher number of blood draws during follow-up was associated with slightly shorter half-lives of both RSV-specific and IgG1 antibodies. This could reflect modest antibody loss due to repeated sampling or indicate that infants requiring more frequent blood tests had greater disease severity, potentially leading to increased antibody catabolism.

An important assumption that shaped both our study design and our quantification of antibody half-life was that passively acquired IgG declines in an approximately exponential fashion, as suggested by previous work [30,31]. Based on this assumption, the difference in antibody levels between two time points should be sufficient to estimate half-life, and we adopted this strategy to minimize participant burden. Consistent with this assumption, we modeled decay as exponential and expressed results in terms of half-life. While this is standard practice, if decay depends on

antibody levels, as our results suggest, half-lives may not be constant. In such cases, estimates will vary with cord blood titers and the interval between blood samples, reducing generalizability and interpretability of our estimated half-lives. Interestingly, if the half-life is dynamic and depends on the antibody concentration at a given time, one would expect the antibody levels of preterm and full-term infants to converge even more rapidly (i.e., resulting in a smaller vaccine gap) than what we modelled in this study.

Our second key assumption was that the relationship between gestational age and cord blood antibody titers follows an exponential form. Although this function captured the overall trend, it may oversimplify biological variability and affect estimates of residual titers and their influence on half-life. In addition, our simulations on vaccine-acquired antibodies assumed that maternal vaccination does not alter this relationship. Early findings from the RSV preF maternal vaccine trial suggest that antibody transfer may be more efficient in vaccinated pregnancies [6]. If so, our model may underestimate cord blood titers in preterm infants and consequently overestimate differences in half-lives between preterm and full-term infants.

If antibody breakdown is concentration-dependent, similar principles may extend to monoclonal antibodies. Yet this factor is rarely incorporated into pharmacokinetic models. A review of pharmacokinetic modeling studies found that although only 11 out of 173 publications evaluated the effect of baseline IgG levels on mAbs clearance, while more than half of these reported a significant positive association [28]. Published data on RSV monoclonal antibodies show shorter half-lives at higher doses [33,34], although this has not been explicitly highlighted in the literature.

These findings may have practical implications at different levels. For maternal vaccines, not accounting for the concentration–catabolism effect could lead to overestimates of the protection gap between preterm and term infants. For monoclonal antibodies, administering several lower doses may be more effective in maintaining protection than a single high dose. However, because our data are based on unvaccinated neonates, further validation in vaccinated cohorts is needed. Future work should investigate how both the concentration and quality of antibody responses influence half-life, particularly in the context of maternal immunization. A clearer understanding of these mechanisms is crucial for optimizing strategies to protect infants against RSV.

## CONTRIBUTIONS AND DISCLOSURES

*Author contributions*

JEW, EP, and LB conceptualized the study. KB and FG provided scientific advice. EP and JGW supervised data collection. JD and JB advised on statistical analyses. LJA, BH, and RS conducted the laboratory work. JEW performed the statistical analyses and wrote the first version of the manuscript. All authors provided critical feedback and approved the final manuscript. JEW, EP, JGW, and LB had full access to the data. LB had final responsibility for the decision to submit for publication.

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*Sponsor*

This study was funded by Pfizer.



## SUPPLEMENTARY MATERIALS

S1.2 Statistical Analysis and S2.1 Clinical impact estimation are provided at the end of this chapter, following the references.

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## SUPPLEMENTARY MATERIALS

### S1.2 Statistical analysis

For each infant, the cord blood titer ($CBTiter_{ij}$) was defined as the cord blood titer at birth or, if unavailable, the first follow-up measurement (obtained within two weeks postnatally). Antibody levels were $\log_2$-transformed and expressed as fold-change relative to their first measurement, so that each infant's baseline was set to zero. On this log2 scale, a decrease of 1 unit corresponds to a halving of antibody levels.

We modeled the $\log_2$ fold-change in RSV-neutralizing antibody (RSV NAb) titers over time using linear mixed-effects models. Specifically, we used the expression: $log_2(\frac{Titer_{ij}}{CBTiter_i})$. With this transformation each individual's trajectory starts at zero, allowing the model to focus solely on estimating the rate of antibody decline (i.e., the slope), independent of their cord blood antibody titer (the intercept).

In this context, the decay rate reflects the daily rate of decline in $\log_2$-transformed titers. More negative values correspond to faster antibody decay. The model was specified as follows:

$$log_2(\frac{Titer_{ij}}{CBTiter_j}) = \beta_1 \cdot Time_{ij} + \beta_2 \cdot (Time_{ij} \times GAc_j) + (b_{1j} \cdot Time_{ij}) + \varepsilon_{ij}$$

Here, $Titer_{ij}$ is the titer for infant j at time point i, and $CBTiter_j$ is that infant's initial (cord blood) titer. Time is expressed in days since the initial measurement. Gestational age was centered at 40 weeks and is denoted as $GAc_j$. The fixed effect $\beta_1$ captures the average decay rate, $\beta_2$ estimates how the decay rate changes with gestational age, $b_{1j}$ is a participant-specific random slope, and $\varepsilon_{ij}$ is the residual error. Residual errors were assumed normally distributed with mean zero and constant variance, and random slopes were assumed normally distributed with mean zero; variance components were estimated using restricted maximum likelihood (REML).[8,9]

Each infant's individual decay rate ($r_j$) was calculated as: $r_j = \beta_1 + \beta_2 \cdot GAc_j + b_{1j}$. From this, the antibody half-life for each infant was derived as: $t_{1/2,j} = -1 / r_j^{\,2}$. This approach allowed us to estimate antibody decay rates and half-lives while accounting for both gestational age and individual variability.

*Residualized cord blood titers as a mediator of decay*
To explore this, we first modeled cord blood titers as a function of gestational age:

$$log_2\left(CBTiter_j\right) = \alpha_0 + \alpha_1 \cdot GAc_j + \varepsilon_j$$

This model was used to estimate each infant's expected cord blood titer ($\widehat{CBTiter_j}$) based on gestational age:

$$log_2\left(\widehat{CBTiter_j}\right) = \alpha_0 + \alpha_1 \cdot GAc_j$$

We defined the residual cord blood titer ($Res_j$) for each infant as the $\log_2$-transformed ratio of the observed cord blood titer to the expected cord blood titer based on gestational age:

$$Res_j = log_2\left(\frac{InitialTiter_j}{\widehat{CBTiter_j}}\right)$$

This residual reflects how much higher or lower an infant's titer was compared to what would be expected for their gestational age. On the $\log_2$ scale, a 1-unit difference in $Res_j$ corresponds to a two-fold difference in titer.

To assess the impact of this residual on the decay rate, we extended our linear mixed-effects model by including an interaction between time and the residual cord blood titer:

$$log_2\left(\frac{Titer_j}{CBTiter_j}\right) = \beta_1 \cdot Time_{ij} + \beta_2 \cdot (Time_{ij} \times GAc_j) + \beta_3 \cdot (Time_{ij} \times Res_j) + (b_j \cdot Time_{ij}) + \varepsilon_{ij}$$

Here, $\beta_3$ estimates how deviation from expected cord blood titers (corrected for gestational age) influences the decay rate.

Each infant's individual decay rate ($r_j$) was calculated as: $r_j = \beta_1 + \beta_2\, GAc_j + \beta_3\, Res_j + b_{1j}$.

Antibody half-lives were derived from these decay rates as: $t_{1/2,j} = -1 / r_j$.

To evaluate whether adding gestational age and residual cord blood titer to the model improved model fit and explanatory power, we performed model selection using likelihood ratio tests. Additionally, we evaluated whether other covariates could help explain variability in antibody decay beyond gestational age and residual cord blood titers. Postnatal exposures such as breastfeeding and repeated blood sampling could theoretically influence measured antibody levels, either by providing exogenous antibodies or by lowering circulating concentrations through frequent venipuncture.

To test these possibilities, we added breastfeeding status and the number of blood draws during hospitalization to the mixed effects model for RSV antibody decay.

All analyses were performed using R version 4.4.0 [3]. Data manipulation and preparation were conducted with the dplyr package [4] (version 1.1.4) and linear mixed-effects models were fitted using the lme4 package [5] (version 1.1.35.3). P-values for fixed effects were obtained using the lmerTest package [6] (version 3.1.3), which applies Satterthwaite's approximation to estimate denominator degrees of freedom. Standard errors and 95% confidence intervals for derived quantities (e.g., antibody half-lives) were calculated using the delta method, implemented via the "deltaMethod" function in the car package [7] (version 3.1.2). Data visualization was performed using ggplot2 [8] (version 3.5.1).

## S2.1 Clincal impact estimation

We examined how gestational age (GA) and cord blood antibody levels influence the persistence of maternally acquired RSV protection. Infants were grouped in 2-week GA intervals from 24 to 42 weeks, and we focused on realistic antibody patterns by limiting analyses to the range actually observed in our cohort. Within these ranges, we estimated how quickly antibodies declined and derived their half-life in days.

To explore the potential impact of maternal vaccination, we also modeled scenarios where cord blood titers were increased beyond what we observed. Because a universally accepted correlate of RSV protection is not yet established [9,10], we used a threshold suggested by Walsh et al. [11]. Walsh et al. have provided a potential biomarker for efficacy by relating vaccine-elicited serum neutralizing titers to those associated with protective palivizumab levels (100 µg/mL) in high-risk infants. In their analysis, a Pfizer neutralization titer of 903 corresponded to a 100 µg/mL palivizumab serum concentration. As recommended by Walsh et al., applying a conversion factor of 0.377 yields 340 IU/mL in WHO International Units. We then estimated the time required for vaccine-induced titers to fall below this protective threshold for each simulated combination of gestational age and cord blood titer.

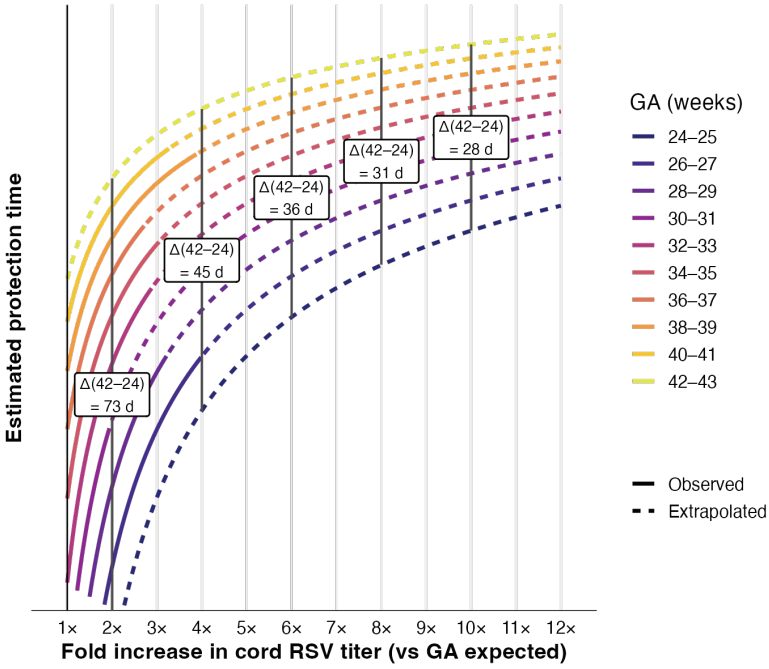
$$T_{prot,j} = \frac{\log\left(\frac{Threshold}{CBTiter_j}\right)}{r_j * \log(2)}$$

To assess the clinical implications of gestational age, cord blood antibody titers, and their rate of decline, we first examined only the range of antibody levels actually observed in our cohort. Within these realistic combinations, we estimated antibody half-lives across gestational ages (based on the coefficients of the M3, Supplementary Table 4). This shows more premature infants are expected to start with lower titers (**Figure 3A**), but also substantially longer half-lives (**Figure 3B**).

We then extended the simulations to mimic potential vaccine effects by modeling fold increases in cord blood titers beyond those seen naturally. In the natural range, titers varied from one quarter to four times the gestational age–expected value, whereas in the vaccination scenario, we extrapolated up to twelve-fold (based on Phase 1/2 results of the RSVpreF vaccine [11]). Using the protective threshold proposed by Walsh et al. [11], we estimated how long antibodies would be expected to remain above this level.

These simulations showed that, even after accounting for the longer half-lives associated with lower antibody levels in preterm infants, a protection gap remains between late-term and preterm infants (**Supplemental Figure 3**). However, the relative difference in protection reduces as cord antibody titers rise through vaccination. For example, at a 2-fold increase the absolute difference in estimated protection was 73 days, whereas at a 10-fold increase this reduced to only 28 days.

Importantly, we assumed that vaccine-induced antibodies decay at the same rate as naturally acquired antibodies, though factors such as IgG subclass distribution, glycosylation, and FcRn affinity are likely to influence decay kinetics [12,13].




**Supplemental Figure 3.** Impact of cord RSV antibody level relative to the gestational age expected level on cord blood titer, antibody half-life, and duration of protection across gestational ages.

Solid lines show predictions within combinations observed in the data; dashed lines indicate extrapolations beyond that range. Colors denote gestational age in weeks. Y-axis labels are omitted to emphasize overall patterns rather than exact values. Vertical lines between the 24- and 42-week curves, with midpoint labels, indicate the absolute difference in days of protection between infants born at 42 and 24 weeks' gestation. These annotations highlight that the protection gap reduces as cord antibody titers rise, for example through vaccination.

*Note:* Fold change is defined relative to the gestational age specific expected level (fold = $2^{\text{residual}}$, where residual is the difference between observed and expected $\log_2$ titer based on GA).

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# Chapter 7

## Placental histopathology and transplacental transfer of RSV specific neutralizing antibodies

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## ABSTRACT

**Introduction**:

Maternally acquired passive immunity via transplacental antibody transport is crucial for protection of infants against infectious diseases in early life. We examined whether placental histological characteristics impact the transplacental transfer of natural RSV-specific neutralizing antibodies.

**Methods**:

We performed a prospective, observational, cross-sectional study. Pregnant women were enrolled with a 1:1:1 inclusion ratio of extremely preterm born infants (24-32 weeks), moderately preterm born infants (33-36 weeks) and term born infants (37-42 weeks). Cord-maternal ratios (CMRs) for serum neutralizing RSV-A and -B antibodies were analyzed in relation to 16 placental histological characteristics.

**Results**:

In total, 85 mother-infant pairs were available for analysis. Infant gestational age was significantly correlated with CMRs for RSV-A and -B specific antibodies (B = 0.10, p <0.001). Although a few placental characteristics showed an univariate association with CMR the multiple variate model showed that only gestational age was independently related to CMR (B 0.11, p <0.001).

**Conclusion**:

Our study confirms that CMRs increase during gestation. However, variations in placenta histology do not appear to affect transplacental RSV-A and RSV-B antibody transfer. We hypothesize that the placental compensatory capacity and active transport mechanism may play a key role in transplacental antibody transfer.

## INTRODUCTION

Maternally acquired passive immunity via transplacental antibody transport is crucial for protection of infants against infectious diseases in early life until the infant's immune system is fully able to produce antibodies itself.[1–3] Maternal immunoglobin G (IgG) is the only immunoglobulin class actively transported over the placenta and the primary source of IgG in umbilical cord blood. [4–7] The process of transplacental transfer starts early in pregnancy, usually around the 13th week of gestation. The fetal IgG levels increase gradually as gestational age increases. At term, fetal IgG levels can even exceed maternal IgG levels at birth.[8]

Maternal IgG antibodies are transported via an active, receptor-mediated process and cross three placental layers to reach the fetal circulation 1) the trophoblast, 2) the intervillous stroma and 3) the fetal vascular endothelium.[9–14] Of these layers, the antibody transfer across the syncytiotrophoblast is most important.[9–11] Briefly, IgG antibodies are endocytosed from extracellular fluid on the maternal side of the syncytiotrophoblast layer. The acidic pH inside the endosome allows binding of IgG to the membrane-bound FcRn (Fragment crystallizable receptor, neonatal). Then, IgG is transcytosed to the fetal surface, where a return to physiological pH allows disassociation of the IgG from the FcRn.[12,15–20] The hypothesis that additional FcγRs (Fragment crystallizable gamma receptors) contribute to transplacental IgG transfer remains a subject of debate.[14] Therefore, based on current knowledge, the FcRn expressed on the placental syncytiotrophoblasts is recognized playing a key role in mediating the transport of maternal IgG across the placenta.[4–6,14]

The placental anatomy supports transplacental transfer of IgG due to the high surface area of syncytiotrophoblasts and extensive (fetal) vascular tree.[14] As gestational age increases, the trophoblasts undergo changes that are closely linked with their capacity for IgG transfer.[14] A dysregulated transplacental transfer can occur in inflamed placenta's. Contributing factors in inflamed placenta's include changes in Fc receptor expression or a disrupted placental development.[14] A disrupted placental development caused by impaired trophoblast and (fetal) vascular development reduces the overall transplacental transfer capacity.[14]

Several other factors influence the efficiency of IgG transplacental transfer, including IgG subclass, glycosylation, maternal infections, and maternal IgG concentration.[20] The maternal IgG concentration can be boosted by maternal vaccination as maternal vaccination induces an enhanced immune response.[21–23] Several countries, including the United Kingdom and Argentina, have now implemented a newly approved maternal vaccine in their immunization programs to protect infants against RSV

disease.[24,25] However, the effect of placental histology on RSV transplacental transfer is currently unknown.

Therefore, an improved understanding of the effect of placental histology on RSV transplacental transfer and subsequently infants RSV immunity is needed. In the current study, we aimed to establish the relationship between naturally occurring transplacental transfer of RSV antibodies and placental histological characteristics. This will deepen our understanding of what factors drive the individual differences in cord to maternal blood titer ratios (CMRs). This may be of importance for the development of new preventive interventions.

## MATERIALS AND METHODS

### *Design*

The SILVER study (Seroepidemiological Longitudinal RSV-specific Antibodies Transfer Study) is a prospective, observational, cross-sectional study performed in the Wilhelmina Children's Hospital, the Netherlands between April 2022 and April 2024. The SILVER study is part of PRISERO, an international seroepidemiology program to assess transplacental transfer of naturally occurring RSV specific neutralizing antibodies. [26]

### *Study population*

Pregnant women were enrolled at the obstetric department of the Wilhelmina Children's Hospital in case they were ≥18 years of age and hospitalized due to imminent labor from 24 0/7 weeks of gestation. We aimed to enroll pregnant women and their (un)born infant with a 1:1:1 inclusion ratio of extremely preterm born infants (≥24 to 31 weeks), moderately preterm born infants (≥32 to 36 weeks) and term born infants (≥37 to 42 weeks). Mother-infant pairs were excluded in case the specimen collection of a mother-infant pair failed <24 hours after birth. In the case of twin births, participants were excluded when the placentas were sent to the pathologist unmarked and could therefore not be linked to the corresponding infant.

### *Data and sample collection*

Data on transplacental transfer at birth was collected by taking cord- and maternal blood samples at birth by obstetric professionals or research personnel. After clamping the umbilical cord and sampling of cord blood the placenta was collected and stored by the obstetric healthcare professionals. Table S1 shows an overview of the collected study samples for mother-infant pairs.

In addition, we collected relevant clinical data from medical records. Maternal data included comorbidities (e.g. immunodeficiency), received corticosteroids, blood products, monoclonal antibodies or Rho(D) immunoglobulin, the delivery method and number of infants born. Additionally, data on received maternal vaccinations was retrieved from mothers. Infant medical variables collected were birthweight and gestational age at birth.

### *Sample testing*

All blood samples were collected in SST vacutainers, transported at 2 to 8 degrees Celsius to the laboratory where it was processed for plasma as soon as possible, with a maximum of 72 hours after administration. Subsequently, the plasma was divided into aliquots and stored at -80 degrees Celsius for later testing. Specimens were tested for functional RSV antibodies. RSV-A and -B specific neutralizing antibodies were measured using a fluorescence-based micro-neutralization assay. Neutralizing titers were calculated using a modified Spearman-Karber method. The detailed methodology has been described by Hotard et al [27] and Anderson et al [28].

### *Placental examination and grading*

After collection, the placenta was sent to the pathology department of the University Medical Centre (UMC) Utrecht where it was stored at +4°C and processed within 72 hours. The following samples were collected and stained with standard haematoxylin and eosin: 2 sections of the umbilical cord: at the fetal and placental side, 1 membrane roll, 1 sample from the umbilical cord insertion, 3 slides of normal placental parenchyma including both decidua and chorionic plate.

An experienced pathologist (PN), who was blinded to clinical information and CMRs, defined the presence and degree of chorioamnionitis, funisitis and villitis using definitions (**Table 1**) from our previous work [29]. Post-hoc analyses were performed to assess other placental histological characteristics according to definitions and the grading system reported in Nikkels et al 2019 [30], Kong et al 2016[31] and Yee Khong et al 2019 [32].

### *Ethical approval*

The study was conducted in compliance with the protocol and the International Conference on Harmonisation Guideline on Good Clinical Practice E6 (ICH-GCP). The study was approved by the Medical Ethics Committee (number 21-836/A). Written informed consent was obtained from all study participants.

**Table 1.** Definition and grading of placental histological characteristics.

| | Histological characteristic | Definition |
|---|---|---|
| Placental disc - Macroscopic | Fetal vascular malperfusion | Groups of at least 5 avascular fibrotic villi, thrombosis in a vessel of the umbilical cord, chorionic plate or stemvillus, haemorrhagic endovasculitis, or intramural deposition of fibrin in a vessel of the umbilical cord, chorionic plate or stemvillus. |
| | Grade 1 | One focus |
| | Grade 2 | Several foci |
| | Grade 3 | Multiple foci in 2 or more slides present |
| | Fibrinoid depositions | Minor and focal fibrinoid depositions not meeting the criteria for massive perivillous fibrinoid deposition (involvement of >50% parenchyma). |
| | Grade 1 | Mild/patchy |
| | Grade 2 | Moderate/ Increased (>25%) |
| | Grade 3 | Severe/ Massive (>50%) |
| | Infarctions | An area of ischemic coagulative necrosis of the placental parenchyma. |
| | Grade 1 | Infarctions in ≤ 5% of placental parenchyma |
| | Grade 2 | Infarctions in >5% of placental parenchyma |
| Placental disc – microscopic | Acute chorioamnionitis | The presence of polymorphonuclear cells (neutrophilic granulocytes, PMN) in the chorionic plate or the extraplacental membranes. |
| | Grade 0.5 | Sporadic PMN in chorionic plate/membranes |
| | Grade 1 | Frequent PMN in chorionic plate/membranes |
| | Grade 2 | Invasion of chorionic plate, large infiltrate in chorionic plate/membranes |
| | Grade 3 | Same as 2, including micro-abscesses in chorionic plate |
| | Chronic chorioamnionitis | An infiltration of lymphocytes and macrophages in the chorionic plate or extraplacental membranes in clusters of at least 50 cells. |
| | Grade 1 | Presence of chronic chorioamnionitis |
| | Delay in the formation of syncytiocapillary membranes (DVM) | Delay in the formation of syncytiocapillary membranes (SCM) in terminal villi. A SCM was defined as a dilated capillary in broad contact with the thin trophoblastic cover, without presence of a nucleus in the contact area according to Stallmach and others. Formation of SCM or maturation was classified as abnormal if in at least one slide the mean of SCM was less than 0.25 SCM/villus in the 10 smallest villi in 10 fields. |
| | Grade 1 | Focal in one slide |
| | Grade 2 | Diffuse in 2 or more slides present |
| | Accelerated villous maturation (AVM) | AVM is defined as the presence of small or short hypermature villi for gestational period. |
| | Grade 1 | Presence of AVM |
| | Distal villous hypoplasia (DVH) | DVH is the paucity of terminal villi with an apparent increase in intervillous space. The total number of villi is decreased in the center of the placenta, and the terminal villi are smaller, elongated, and slender compared with normal terminal villi. The diagnosis should be made when the features are seen in the lower two-thirds (not subchorionic plate) and involve at least 30% of a full-thickness slide (chorionic plate to the decidua basalis). It may be further graded as focal finding of the lesion in one full-thickness slide only or diffuse if present in two or more full-thickness slides. |
| | Grade 1 | Focal |

| | | |
|---|---|---|
| | Grade 2 | Generalized |
| | Nucleated red blood cells (NRBCs) | The presence of NRBC in terminal villi respectively. |
| | Grade 1 | Sporadic presence of NRBC |
| | Grade 2 | Frequently easy to find NRBCs |
| | Grade 3 | Many NRBCs |
| | Necrosis in decidua | Band of coagulative necrosis in decidua with or without leucocytoclastic reaction. |
| | Grade 1 | Presence of necrosis in decidua |
| | Villitis | An infiltration of lymphocytes and macrophages in the placental villi. |
| | Grade 1 | 1 section with 1 focus of chronic inflammation of >5 villi |
| | Grade 2 | 2–3 sections with each 1 focus of chronic |
| | Grade 3 | 3 sections with each >2 foci of chronic |
| | Chorangiosis | Diffuse or patchy distribution of 10 or more villi containing 10 or more capillary cross sections in terminal or intermediate villi. In addition, in some villi 15-20 capillary cross sections should be seen to prevent overdiagnosis. |
| | Grade 1 | Local |
| | Grade 2 | Diffuse |
| | Ischemic changes | Ischemic changes/increased occurrence of syncytial knots: more than 1/3 of affected villi per slide. Focal in one slide and diffuse in 2 or more slides present. |
| | Grade 1 | Focal in one slide |
| | Grade 2 | Diffuse in two or more slides present |
| **Umbilical cord** | Funisitis | The presence of neutrophils in the wall of the umbilical vein and/or arteries or Wharton's jelly. |
| | Grade 1 | Inflammation present in the wall of 1 vessel (vein) |
| | Grade 2 | Inflammation present in the wall of 2 vessels (vein and artery) |
| | Grade 3 | Inflammation present in the wall of 3 vessels (vein and 2 arteries) |
| | Umbilical coiling index (UCI) | The number of coils divided by length of the umbilical cord. |
| | Hypocoiling | UCI below the 10th percentile (<0.07) |
| | Hypercoiling | UCI above the 90th centile (>0.30) |
| **Gross examinatio** | Placental weight | The weight of the placental disk rinsed clean of loose blood and trimmed at the margin to remove the extraplacental membranes and the umbilical cord at its insertion into the chorion. |
| | Low for GA | A placental weight below the 10th percentile. |
| | High for GA | A placental weight above the 90th percentile. |

*Statistical analysis*

In this study we aimed to determine the effect of the placental histology on the transplacental transfer. To quantify this transfer, we used the cord-maternal ratio (CMR) of neutralizing RSV antibodies at birth. CMRs were calculated by dividing the neutralizing antibody titers in cord blood, by the neutralizing antibody titers in paired maternal blood.

*Data preparation*

Before analysis, presence of outliers was evaluated by visual inspection of histograms and Stem-and-Leaf plots and critically assessed and being retained if the CMR was judged realistic.

Since RSV-A and RSV-B CMRs reflect the same underlying mechanism of transplacental transfer capacity, we first assessed their correlation as a measure of reliability. We then calculated the geometric mean of the CMR ratio for RSV-A and RSV-B for each mother-infant pair, by using the square root of their product. The geometric means of CMRs were subsequently log2 transformed to approximate a normal distribution. Furthermore, the placental histological characteristics were regrouped: when the most severe category was present in fewer than 5% of mother–infant pairs, the two highest severity levels were merged to reduce sparse categories and minimize the risk of overfitting. Lastly, birthweights were transformed to percentiles using gestational age in completed weeks from the Dutch birthweight charts of Hoftiezer et al[33]. Subsequently, small for gestational age was defined as a birthweight below the 5$^{th}$ percentile.

*Main analysis*

To evaluate the individual impact of placental histological characteristics, gestational age and small for gestational age on CMRs, we performed univariate linear regression analyses, with each variable entered separately as a predictor of CMR. Subsequently, the joint effects of placental characteristics on CMRs were assessed using multiple linear regression with backward selection, including gestational age and small for gestational age. Variables were sequentially removed according to the default SPSS criteria (probability of F-to-remove = 0.10). Regression parameters and 95% confidence intervals are reported (**Table 2**). All analyses were conducted in SPSS (Version 29.0.2.0).

*Sensitivity analysis*

The CMR offers a direct measure of antibody transfer mediated by placental function. However, this ratio is mathematically unstable because it involves dividing two measurements, each subject to measurement error. As a more mathematical robust alternative, cord blood antibody titers can be used. While this method provides a less direct assessment of placental antibody transfer, it is less affected by the compounding effects of measurement variability. Therefore, we considered cord blood antibody titer as an alternative measure for transplacental transfer in a sensitivity analysis. The procedure was repeated with calculation of the geometric mean based on cord blood concentrations of RSV-A and RSV-B instead of ratios and subsequently a backward linear regression was performed.

## RESULTS

*Baseline*

In total, 109 mother-infant pairs with data on placental histology were available. Of these mother-infant pairs, 24 (22%) were twin births with unmarked placentas (i.e. placentas not clearly assigned to an individual infant) leaving 85 mother-infant pairs for analysis (**Figure 1**).

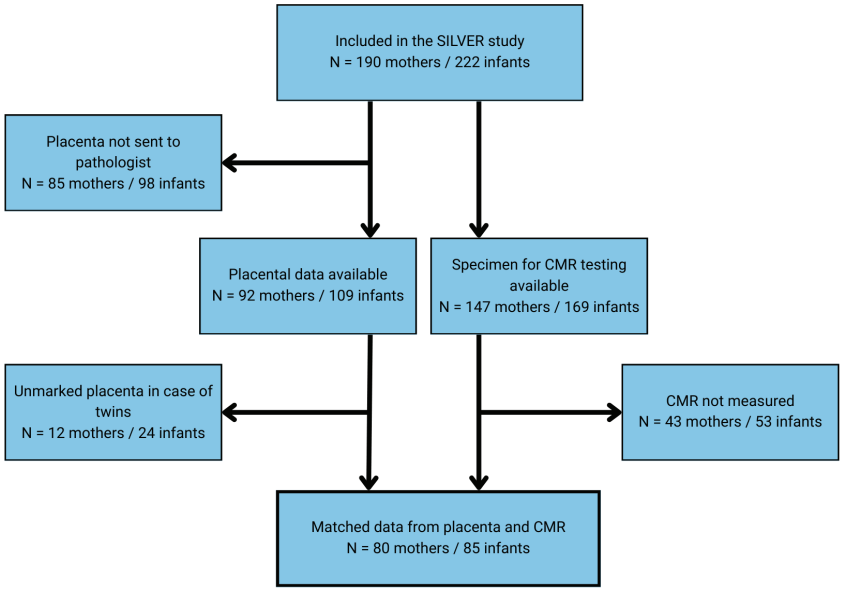



**Figure 1.** Flowchart of study participants.

All CMRs were judged realistic. We included 34 (40%) extremely preterm births, 18 (21.2%) moderately preterm births and 33 (38.8%) term births. Descriptive characteristics are presented in **Table 2**. Most infants were born via caesarean section (C-section). Gestational age at delivery was lower in C-sections (median 33.5 weeks) compared to vaginal deliveries (median 38.0 weeks, p 0.02). Small for gestational age was observed in 17 (20.0%) infants but was not correlated with CMRs. Infant gestational age was significantly correlated with CMRs and cord blood concentrations for RSV-A and -B specific antibodies (p <0.001; **Table 3**).

**Table 2.** Descriptive characteristics of all mother-infant pairs.

| Characteristics | Result |
|---|---|
| N | 85 mother-infant pairs |
| Maternal risk factors | |
| Auto-immune disease | - |
| Immunodeficiency | - |
| HIV positive | - |
| Hypertension* | 7 (8.2%) |
| Pre-eclampsia | 3 (3.5%) |
| HELLP syndrome | 1 (1.2%) |
| Gestational age categories | |
| Extremely preterm (24-32 weeks) | 34 (40.0%) |
| Moderately preterm (33-36 weeks) | 18 (21.2%) |
| Full term (37-42 weeks) | 33 (38.8%) |
| Small for gestational age (< 5$^{th}$ percentile) | 17 (20.0%) |
| Singleton births | 75 (88.2%) |
| Twin births | 10 (11.8%) |
| Monochorionic-monoamniotic | - |
| Monochorionic-biamniotic | 4 (4.7%) |
| Bichorionic-biamniotic | 6 (7.1%) |
| Mode of delivery | |
| Vaginal | 37 (43.5%) |
| Cesarean section | 48 (56.5%) |

HIV = human immunodeficiency virus, HELLP = hemolysis elevated liver enzymes low platelets.

*Hypertension also includes pre-eclampsia and HELLP syndrome.

**Table 3.** Results of univariate analysis of gestational age on CMRs and cord blood titers.

| Outcome | Coefficient | | Standardized coefficient β | p-value | Adjusted $R^2$ |
|---|---|---|---|---|---|
| | Regression coefficient B | 95% CI | | | |
| RSV-A specific CMR | 0.064 | 0.030;0.098 | 0.378 | <0.001 | 0.132 |
| RSV-B specific CMR | 0.144 | 0.105;0.182 | 0.633 | <0.001 | 0.393 |
| Geometric mean of CMRs | 0.104 | 0.073;0.135 | 0.587 | <0.001 | 0.337 |
| RSV-A specific CB titer | 0.124 | 0.073;0.174 | 0.473 | <0.001 | 0.215 |
| RSV-B specific CB titer | 0.209 | 0.147;0.271 | 0.592 | <0.001 | 0.342 |
| Geometric mean of CB titers | 0.166 | 0.121;0.211 | 0.629 | <0.001 | 0.389 |

CMR = cord-maternal ratio, CB = cord blood.

*Placenta histology*

Most frequently observed placental histological characteristics were ischemic changes (n=38, 44.7%), infarctions (n=32, 37.6%), nucleated red blood cells (NRBCs) (n=29, 34.1%) and a small for gestational age placental weight (n=28, 32.9%). The presence and grading of all placental anomalies is reported in **Table 4**.

To evaluate the individual impact of placental histology on CMRs, we performed univariate analysis for each indicator (**Table 5**). The presence of accelerated villous maturation (AVM) and NRBC were negatively correlated with CMR (B = -0.53 and B = -0.32 respectively, p 0.006), whereas the presence of delayed villous maturation (DVM) was positively correlated with CMR (B = 0.50, p 0.02). Our multivariate regression model demonstrated that gestational age was the only independent variable significantly associated with CMR (B = 0.11, $p < 0.001$) (Table S2).

*Sensitivity analysis: placental histology and their effect on cord blood titers*

In the sensitivity analysis, the final model demonstrated that, besides the positive effect of gestational age, fetal vascular malperfusion decreases RSV cord blood titers (B = -0.957, p 0.004) (Table S3).

**Table 4.** Presence and grading of placental histological characteristics.

| Characteristic | Result (n = 85) |
|---|---|
| Fetal vascular malperfusion [a] | 9 (10.7%) |
| Grade 1 | 8 (9.5%) |
| Grade 2 | - |
| Grade 3 | 1 (1.2%) |
| Fibrinoid depositions | 7 (8.2%) |
| Grade 1 | 7 (8.2%) |
| Grade 2 | - |
| Grade 3 | - |
| Infarctions | 32 (37.6%) |
| Grade 1 | 28 (32.9%) |
| Grade 2 | 4 (4.7%) |
| Acute chorioamnionitis | 19 (22.4%) |
| Grade 1 | 4 (4.7%) |
| Grade 0.5 | - |
| Grade 2 | 6 (7.1%) |
| Grade 3 | 9 (10.6%) |
| Chronic chorioamnionitis | 2 (2.4%) |
| DVM | 18 (21.2%) |
| Grade 1 | 17 (20.0%) |
| Grade 2 | 1 (1.2%) |
| AVM | 25 (29.4%) |
| DVH | 5 (5.9%) |
| Grade 1 | 3 (3.5%) |
| Grade 2 | 2 (2.4%) |
| NRBC | 29 (34.1%) |
| Grade 1 | 16 (18.8%) |
| Grade 2 | 12 (14.1%) |
| Grade 3 | 1 (1.2%) |
| Necrosis in decidua | 1 (1.2%) |
| Villitis | 18 (21.2%) |
| Grade 1 | 14 (16.5%) |
| Grade 2 | 3 (3.5%) |
| Grade 3 | 1 (1.2%) |
| Chorangiosis | 10 (11.8%) |
| Grade 1 | 7 (8.2%) |
| Grade 2 | 3 (3.5%) |
| Ischemic changes | 38 (44.7%) |
| Grade 1 | 23 (27.1%) |
| Grade 2 | 15 (17.6%) |
| Funisitis | 10 (11.8%) |
| Grade 1 | 3 (3.5%) |
| Grade 2 | 1 (1.2%) |
| Grade 3 | 6 (7.1%) |
| Hypocoiling[a] | 23 (27.4%) |
| Hypercoiling[a] | 4 (4.8%) |
| Placental weight low for GA | 28 (32.9%) |
| Placental weight high for GA | 18 (21.2%) |

DVM = delayed villous maturation, AVM = accelerated villous maturation, DVH = distal villous hypoplasia, NRBC = nucleated red blood cells, GA = gestational age.
[a] Data on fetal vascular malperfusion was missing for one participant and data on the coilingindex was missing for one participant.

**Table 5.** Results of univariate analysis of individual placental histological characteristics and gestational age on CMRs.

| Characteristic | Coefficient | | Standardized coefficient β | p-value |
|---|---|---|---|---|
| | Regression coefficient B | 95% CI | | |
| Gestational age | 0.104 | 0.073;0.135 | 0.587 | **<0.001** |
| Small for gestational age | -0.174 | -0.617;0.270 | -0.085 | 0.438 |
| Fetal vascular malperfusion | -0.304 | -0.881;0.273 | -0.115 | 0.298 |
| Fibrinoid depositions | 0.183 | -0.464;0.829 | 0.062 | 0.575 |
| Infarctions | 0.112 | -0.193;0.417 | 0.080 | 0.466 |
| Acute chorioamnionitis | -0.095 | -0.269;0.080 | -0.118 | 0.283 |
| Chronic chorioamnionitis | 0.958 | -0.198;2.114 | 0.178 | 0.103 |
| DVM | 0.505 | 0.083;0.926 | 0.253 | **0.020** |
| AVM | -0.534 | -0.907;-0.161 | -0.299 | **0.006** |
| DVH | -0.433 | -1.183;0.318 | -0.125 | 0.255 |
| NRBC | -0.322 | -0.550;-0.094 | 0.294 | **0.006** |
| Necrosis | 0.546 | -1.101;2.193 | 0.072 | 0.512 |
| Villitis | 0.004 | -0.329;0.337 | 0.003 | 0.982 |
| Chorangiosis | 0.048 | -0.505;0.600 | 0.019 | 0.863 |
| Ischemic changes | -0.084 | -0.315;0.148 | -0.079 | 0.473 |
| Funisitis | -0.210 | -0.427;0.007 | -0.207 | 0.057 |
| Hypocoiling | -0.259 | -0.657;0.139 | -0.141 | 0.200 |
| Hypercoiling | -0.480 | -1.315;0.356 | -0.125 | 0.257 |
| Low placental weight | -0.215 | -0.591;0.161 | -0.124 | 0.258 |
| High placental weight | -0.035 | -0.471;0.401 | -0.017 | 0.874 |

CMR = cord-maternal ratio, CI = confidence interval, DVM = delayed villous maturation, AVM = accelerated villous maturation, DVH = distal villous hypoplasia, NRBC = nucleated red blood cells.

## DISCUSSION

This study assessed whether variations in placental histology are related to CMRs. In this study we confirmed that the transplacental transfer of maternal IgG increases during gestation.[8,15,34] We hypothesized that placental histopathology impairs the transplacental transfer of RSV neutralizing antibodies. However, we did not find evidence that the placental histology impacts the transplacental transfer of RSV-specific neutralizing antibodies.

We hypothesize that the placental compensatory capacity, may explain our findings. The placental compensatory capacity depends on the placental weight and the background morphology.[12,35] As gestational age increases, the trophoblasts undergo structural changes, the placental surface area expands and the expression of the FcRn receptor increases.[12,14] Since placental anomalies may have formed at different stages throughout gestation, this may result in differing abilities of the placenta to compensate.[31] Furthermore, we hypothesize that, in contrast to passive diffusion of oxygen and nutrients, the active transport of maternal antibodies across the placenta ensures the infants passively acquired immunity. For example, fetal growth restriction is a fetal comorbidity associated with specific placental anomalies and differences in transplacental transfer.[32,36,37] However, in our study small for gestational age was not correlated with CMRs. This is in line with a recent study showing that, after adjusting for prematurity, the RSV specific antibody transfer was not impaired in infants with low birthweight.[38] Also, some studies indicate that maternal virus infections (e.g. zika virus, dengue virus, cytomegalovirus) impair transplacental oxygen and nutrient transfer[39]. However, the effect of these viral infections on the syncytiotrophoblast and subsequent villous maturation and antibody transfer is poorly understood.

Future research is necessary to examine the impact of variations in placental histology in high-risk populations, for example with maternal comorbid conditions, especially those affecting the placenta. We hypothesize that due to the placental compensatory capacity, an impaired transplacental antibody transfer is not driven by a single factor, but by the cumulative interaction of multiple factors. Considering the cumulative effect of placental histological characteristics and clinical factors might help to better understand the potential clinical significance. However, to better understand the placental capacity to compensate for histopathological characteristics, future research should comprehensively characterize “healthy” placentas throughout gestation first.[32] Yet, this remains a major gap in knowledge.

Our study has multiple strengths. To the best of our knowledge, this is the first study that assessed the relationship between individual differences in CMRs and placental histology. First, all placentas were examined by an experienced pathologist from the UMCU. UMCU has major experience with this line of placental research.[29] In addition, we

analyzed samples on neutralizing RSV-specific antibodies, to examine if the placental histology potentially impacts the protection against RSV infection. Also, we limited potential batch effects by analyzing serum samples from each mother–infant pair within the same batch. Furthermore, our cohort comprises very preterm infants and spans the entire range of gestational ages. In addition, the clinical variables are extracted from electronic medical records, resulting in a high accuracy of the included variables. Our study also has several limitations. First, although we were able to include an appropriate cohort of mother-infant pairs, mostly mild degrees of placental anomalies were found in the examined placentas, which limited our ability to detect a significant effect. Second, participants in our study resided in a high-income setting, in a study population consisting predominantly of mothers without comorbid conditions that are known to affect transplacental antibody transfer, such as malaria or HIV infection. We only found hypertensive disorders in 8.2% of the participants. However, we performed a post-hoc analysis showing no statistical difference in CMRs from mothers with or without hypertensive disorders in our study. Third, our study was performed in an academic medical center with a high incidence of high-risk pregnancies. Consequently, C-sections were the mode of delivery in more than half or the participants in our study. In the Netherlands the general incidence of C-sections is only 18%.[40] In addition, half of the C-sections in our study were urgent indicating an acute problem rather than a consistent impairment of placental efficiency. Fourth, given the number of variables assessed in the univariate and multivariate analyses, the possibility of type I error cannot be excluded. Fifth, we cannot directly assess the impact of RSV dynamics in our study. We evaluated both RSV-A and RSV-B CMRs. Although RSV-A and RSV-B showed similar patterns and CMRs, the maternal and cord blood titers for RSV-B were higher compared to RSV-A. During our enrolment period RSV-B was the dominant subtype, and in the last three months of enrolment this shifted to RSV-A dominance.[41–43] Lastly, several immunological factors may influence the transplacental transfer capacity, including IgG subclass, glycosylation patterns and affinity to Fc receptors. [20] However, our study aimed to measure the effect of placental histology on the neutralizing capacity of RSV-specific antibodies, rather than the precise levels of RSV immunoglobulins, the subclasses and their glycosylation patterns.



In conclusion, our study did not find evidence that variations in placenta histology impact transplacental transfer in preterm and term born infants. While we found effects of individual placental histological characteristics, the final model showed that CMR is correlated with GA only. We hypothesize that due to the placental compensatory capacity, an impaired transplacental antibody transfer is driven by the cumulative interaction of multiple factors. Considering the cumulative effect of clinical factors and placental histological characteristics might help to better understand the potential clinical significance.

## NOTES

*Author contributions*

EP, JW, JGW, LB contributed to conceptualization. KB and FG provided scientific advice. EP, JW, JGW, LB, PN, LA contributed to methodology. EP, JGW contributed to data collection. LA conducted the laboratory work. PN conducted the placental examination. EP, JW, JGW, LB contributed to formal analysis and investigation. EP wrote the first version of the manuscript. All authors contributed to writing-review and editing. All authors contributed to approval of final version. JGW, LB and contributed to supervision.

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# Part IV

## Clinical trial design



"Keep it simple"

- Louis Bont

"De kracht van eenvoud"
- Jose Borghans

# Chapter 8

## Workshop on the design and use of clinical trials with multiple endpoints, with a focus on prevention of RSV

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## Short executive summary

A meeting held in Lisbon, Portugal, in February 2023 focused on critical aspects of clinical trial design for respiratory syncytial virus (RSV) preventative therapies. The meeting addressed two primary areas: enhancing the efficiency and success of randomized controlled trials (RCTs) for RSV preventative therapies and designing RCTs to better inform post-licensure decision-making. Topics included the selection of primary endpoints, innovative approaches to incorporating multiple endpoints and historical data, and the challenges and benefits of sequential trial designs. The discussion also touched on meta-regression models for obtaining more robust, context-specific estimates of vaccine efficacy. Overall, the meeting underscored the importance of balancing efficiency and robustness in RSV vaccine trial design, while recognizing the need for further discussions involving regulatory and advisory bodies.

## BACKGROUND

Randomised, controlled clinical trials are considered the gold standard for evaluating the safety and efficacy of new medical products, including preventive products such as vaccines. However, licensure trials are resource intensive. Bringing a new drug or vaccine to market can be extremely expensive, with estimates ranging from $800 million to $2 billion [1, 2]. Late-stage failures and the rising costs of Phase III trials are major factors contributing to these development costs [3]. Consequently, improving the power and cost-effectiveness of a trial are valuable objectives to pursue, including inclusion of additional experts in design considerations, such as health economists.

When designing a randomized controlled trial (RCT) for regulatory approval, several decisions must be made that can affect its ultimate success and efficiency. These include the choice of the primary clinical endpoint(s), where to enroll subjects, which subjects to exclude, how many subjects to enroll and follow-up, and how to analyze the data. Decisions made when designing a trial are typically meant to maximize the chances of licensure if the intervention is efficacious. However, decision-making can be difficult prior to the start of a trial, especially when knowledge of the incidence of the primary endpoint(s) is uncertain. Also, the selection of primary outcomes for a trial might not be those most relevant for deciding on the public health deployment of the intervention post-licensure, including in low- and middle-income countries (LMICs). Furthermore, trials might not have sufficient evidence within key subgroups or geographic locations, as trials are often not designed to provide the information needed for country-level decision-makers during the post-licensure phase. Many countries have to rely on randomised clinical trial data from other countries, where the outcomes and subgroups studies may have differed from their priorities.

To accelerate clinical development and increase the chances that an efficacious vaccine is approved in a timely fashion, innovative trial designs can be beneficial. Such designs could include incorporating multiple endpoints in the analysis, incorporating data from previous trials into the analysis, and using sequential trial designs with frequent evaluation points [4].

A meeting was held February 21-22, 2023 in Lisbon, Portugal to discuss technical, policy, and implementation issues related to the design of clinical trials, with a focus on preventative therapies against RSV. The meeting covered two general topics: 1) how to decrease the required sample size and increase chances of success of RCTs for safe and efficacious vaccines (by incorporating multiple endpoints in clinical trials, and incorporating historical data from previous trials) and 2) how to design and analyze RCTs to better inform post-licensure decision-making (**Figure 1**). Attendees included representatives from the World Health Organization (WHO), the European Medicines Agency (EMA), the United States Center for Disease Control and Prevention

(CDC), several vaccine manufacturers, the Bill & Melinda Gates Foundation (BMGF), and academic trialists, statisticians, and epidemiologists.

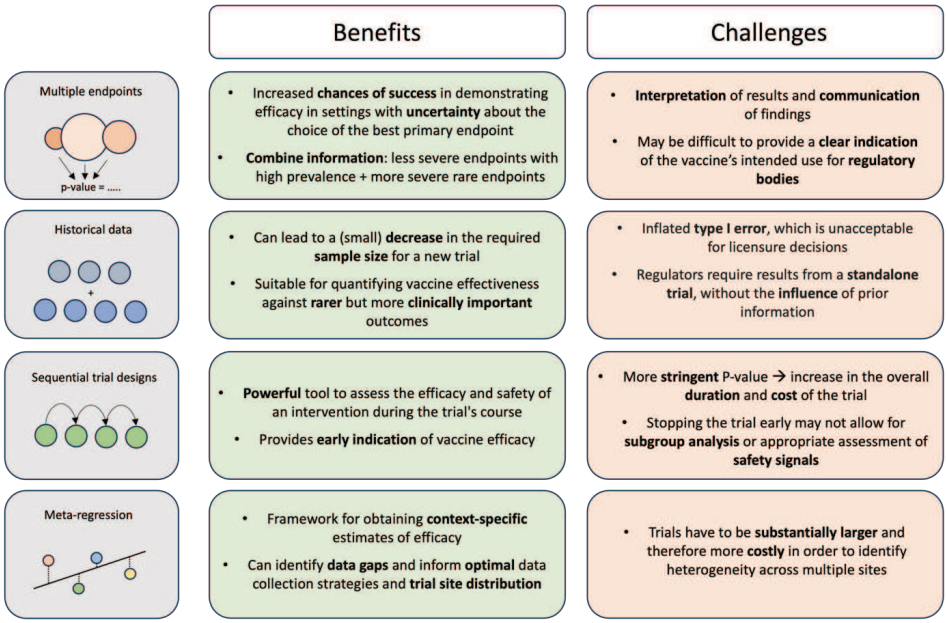



**Figure 1.** Summary of the benefits and challenges of different approaches to attempt making clinical trials more efficient as discussed during the meeting.

## OBJECTIVES OF THE WORKSHOP

The workshop was organized to address three questions with a focus on RSV prevention:

1) How can multiple clinical endpoints be incorporated into an analysis plan?

2) How can data from previous trials (e.g., Phase IIb) be integrated with Phase III results to design more efficient trials?

3) How do we optimally use data from clinical trials with multiple sites to inform decision-making by National Immunization Technical Advisory Groups (NITAGs)?

**Box 1. History of RSV immunization development:**

The development of RSV preventative therapies has been remarkably challenging [5].
A formalin-inactivated vaccine for infants tested in the 1960s increased the severity of RSV disease and caused some deaths, stalling progress for decades. In 1996, RSV-IGIV, an intravenous immunoglobulin preparation, became the first FDA-approved agent for preventing RSV in preterm infants. However, despite initial success, there were safety concerns and logistical issues related to the intravenous administration of a large volume of product and questions about its effectiveness in certain patient groups [6].

A breakthrough occurred in 1998, with the licensure of palivizumab, an anti-RSV monoclonal antibody delivered intramuscularly. Another pivotal moment was the resolution of the RSV fusion (F) protein in its two conformations: the metastable pre-fusion (preF) and the post-fusion (postF) forms. PreF-binding antibodies proved more effective at neutralizing RSV than those targeting postF. This discovery enabled mapping of potent neutralizing epitopes exclusive to the preF form, driving structure-based vaccine design through stabilization of the RSV F protein in the preF conformation [5].

Recently, there have been a string of successes. A Phase III clinical trial of a maternal vaccine targeting preF of RSV showed efficacy in preventing RSV lower respiratory tract infection in infants born to vaccinated mothers, providing protection via passive antibody transfer to the infant [7]; and an extended half-life monoclonal antibody against the preF site was shown to be efficacious in infants [8]; FDA and EMA approval swiftly followed for both products [9-12]. In addition, two vaccines have been shown to be effective and licensed in older adults [13-15], while marketing authorization applications have been submitted for a third investigational vaccine [16].

## INCORPORATING MULTIPLE ENDPOINTS IN CLINICAL TRIALS

In clinical trials designed for obtaining licensure, regulatory agencies require the selection of a primary clinical endpoint, or sometimes two co-primary endpoints depending on the questions to be answered on efficacy or safety. This selection is usually made following scientific advice from the regulatory bodies before the study begins to assess the vaccine's efficacy. Determining which endpoint should be designated as the 'primary' one can be a challenging decision. All potential outcomes under consideration should have clinical relevance, but the definition of clinical relevance may vary among different committees and countries. One potential solution would be to simultaneously consider multiple clinical endpoints.

In the first session of the meeting, we focused on methods for analyzing data on multiple endpoints, comparing the widely applied and most conservative approach, the Bonferroni test, which evaluates each endpoint individually and tests whether any of them are significant, to permutation tests, which account for the correlation between overlapping or nested outcomes [17-20]. We also discussed alternative approaches for integrating multiple endpoints, such as constructing a single composite endpoint (i.e., defined as having outcome A OR outcome B). Throughout the discussions, we examined the limitations, advantages, and extensions of these methods, as well as their possible future applications. Additionally, we discussed the different perspectives and focus of different bodies, e.g. those who license the vaccine and those responsible for decisions about post-licensure deployment of the vaccine, given the criteria for their decisions may be different.

### *Considerations in the choice of a primary endpoint for clinical trials*

A cautionary tale about the influence of which primary endpoint to choose comes from a Phase III clinical trial of a maternal vaccine candidate targeting the prevention of respiratory syncytial virus (RSV) in infants (the Prepare trial) [21]. The vaccine did not demonstrate statistically significant efficacy against the pre-specified primary endpoint of medically significant RSV lower respiratory tract infection (LRTI). This may have been due to an unexpectedly lower incidence of primary endpoint events among the enrolled infants and lack of inclusion of all episodes [21]. As a result, the vaccine fell short of the regulatory threshold for licensure. The efficacy estimate for the primary endpoint was 39.4% with 97.52% confidence interval [CI], −1.0 to 63.7. However, the vaccine demonstrated statistically significant effects against secondary endpoints including hospitalization due to RSV LRTI. During the meeting, it was postulated that the vaccine might have met criteria for licensure had the trial specified composite endpoints (such as medically significant RSV LRTI and/or hospitalization due to RSV LRTI) in its analysis plan or adopted an alternative trial design or analysis strategy.

The selection of trial endpoints must also take into account the need for objective and consistent definitions and measurements of endpoints. This is required by regulators with whom there is pre-trial agreement on the choice of the endpoints. Without standardized definitions, the comparability and reliability of study results can be compromised. This issue becomes particularly challenging when multiple products are under evaluation, recommended, or marketed for the same disease. For instance, the definition of severe lower respiratory tract infection (LRTI) in one study may differ significantly from another study's definition. These discrepancies in definitions can lead to confusions when comparing the efficacy endpoints across studies. Therefore, it is imperative to strive for harmonization and standardization of endpoint definitions across trials and studies. This ensures that the evaluation of different products or interventions is based on consistent criteria, promoting more accurate and meaningful comparisons and assessments within the context of the same disease [22].

From a public health and policymaker standpoint, the choice of endpoints should align with their contribution to the disease burden to enhance the post-licensure deployment of the vaccine. This would mean prioritizing the most severe outcomes, such as hospitalization and death. While higher efficacy is typically expected against more severe disease, these outcomes generally have lower incidence rates compared to less severe endpoints and therefore may provide insufficient statistical power to drive a successful trial, unless the number of participants to be enrolled is increased considerably as well as the cost and potential duration of the trial.

For vaccine manufacturers, the primary goal before licensure is to conduct a successful clinical trial that maximizes the chances of approval. Consequently, trials often focus on less severe endpoints with higher incidence rates to ensure sufficient statistical power within a manageable trial size [8]. For example, the evidence from the GSK RSVPReF3 vaccine trial in older adults was graded stronger for less severe outcomes, like RSV LRTI and medically attended RSV LRTI, than for more severe outcomes, like hospitalization and death due to RSV, according to the Grading of Recommendations, Assessment, Development and Evaluation Summary as presented in an ACIP meeting [23]. The choice of endpoints is often based on the assumption that if efficacy is demonstrated against the more common but milder endpoints, the vaccine is likely to be efficacious against the more severe endpoints which are of greater public health importance, though demonstration of this may await post-licensure effectiveness studies. Several approaches have been developed to design and analyze trials with multiple outcomes [17, 24]. Certain types of multiple endpoint analysis allows the use of both more severe and more common endpoints in the analysis, to decrease the required sample size [25].

*Strengths and limitations of adopting a multiple endpoint approach: considerations from different perspectives*

A common way to incorporate data on multiple endpoints is to create a composite endpoint. For instance, if a subject has outcome A OR outcome B, they are categorized as having the composite outcome. An example is the MAKI trial of the effectiveness of palivizumab for the prevention of asthma, where the composite endpoint was defined as current asthma consisting of both parent-reported wheeze in the past 12 months and/or asthma medication in the past 12 months [26]. An advantage of this approach is that it provides a straightforward way to incorporate data from multiple endpoints of interest, increasing the power of a study, and it is fairly simple to interpret [7]. The reduction in the required sample size is greatest when there is little overlap in disease definitions. However, the conclusions based on composite endpoints can be misleading when there is a difference in frequency of events, clinical importance, or the effectiveness of the intervention on each component of the composite endpoint. Additionally, it does not offer the flexibility to incorporate weighted values that reflect the statistical importance of different outcomes.

Approaches that consider the correlation and overlap between the outcomes can be more powerful than methods that analyze each outcome individually. Typically, permutation-based approaches [20] would provide more weight to outcomes based on their frequency (e.g., inverse-variance weights). However, it was proposed at the meeting that alternative weights could be considered that incorporate viewpoints from different stakeholders about the importance of the endpoints (e.g., based on severity, or based on qualitative priorities of vaccine recipients). Those weights should be pre-specified in the protocol. During the meeting, there was a general agreement that this approach may be more suitable for a Phase IV trial after the vaccine has already been authorised. For example, it might be useful to evaluate the effectiveness of the extended half-life RSV prophylaxis, nirsevimab, or of the maternal RSVpreF vaccine, and to compare performances across countries; both therapeutic preventive strategies showed positive results from the Phase III Melody and Matisse trials, respectively [7, 8].

A challenge related to the use of these methods comes in the interpretation of results and communication of findings to various stakeholders. For example, the permutation-based approach that uses a weighted average to combine different outcomes [25], which was presented during the meeting, might show an overall significant effect of the intervention, even when the effects against individual outcomes are not significant when tested individually in a post-hoc analysis. Although this would benefit the power of the trial, manufacturers and regulatory bodies require primary endpoints to be clinically meaningful and interpretable so that they can develop label indications and appropriately weigh the benefits against risks. The relevance of a measure that integrates multiple outcomes may not be clear, some of which individually might not reach statistical significance in a post-hoc analysis.

In conclusion, the various needs and perspectives of different stakeholders drive the design of clinical trials. The meeting participants agreed that while a single-endpoint approach may be more straightforward for regulatory approval, a multiple-endpoint approach can increase the likelihood of detecting an effect and obtaining approval, if multiplicity issues are also taken into account. By combining multiple endpoints in the analysis, different stakeholders can make informed decisions based on the clinical trial data; a trial can gain power by evaluating non-severe endpoints with higher incidence rates, as well as incorporating information of more severe endpoints while enabling decisions about the intervention's efficacy in public health practice to be made later. However, questions about how the results of analyses that use multiple endpoints would be incorporated into licensure and labeling decisions need to be resolved.

## MAKING TRIAL DESIGNS MORE EFFICIENT

During the second session of the meeting, we focused on two complementary topics: incorporating estimates of vaccine efficacy from previous trials and sequential trial designs.

In general, when conducting a new evaluation of preventative therapies (i.e., active and passive immunization), data from previous trials are ignored beyond support for sample size calculations, despite the potential to provide valuable information. For example, Phase IIb trials may provide evidence of a beneficial but not statistically significant effect of vaccination against clinical endpoints; incorporating data from the Phase IIb trial into the analysis of a Phase III trial could allow for a smaller trial and accelerate the licensure of the vaccine. The Phase III trial could be conducted as a traditional trial with a fixed sample size, or as a group sequential Bayesian trial where vaccine efficacy is evaluated on an ongoing basis and the trial is stopped when sufficient evidence of efficacy has been collected. We discussed limitations and extensions of these approaches. We also considered situations in sequential trials where the decision of whether to continue or stop the trial is unclear



### *Incorporating historical data from previous trials*

A Bayesian method for incorporating data from previous trials was discussed, in which estimates of vaccine efficacy from a previous trial are used to inform the prior distribution estimate for a new trial using the dynamic borrowing prior distributions methods [27]. Including prior information from previous and historical trials into new trials would potentially decrease the required sample size of the new trial and allow for more efficient use of resources. The downside of incorporating historical data from previous trials in new trials is that the use of historical data can inflate the type I error, which is unacceptable for licensure decisions. To correct for this, the threshold on the

type I error should be more stringent, and, consequently, the benefit of incorporating previous evidence is reduced, yielding only a modest reduction in sample size needed for the new trial.

There were a number of important concerns raised about this approach. Regulators may remain unconvinced that a Bayesian trial design offers power advantages over a frequentist design whilst also having appropriate frequentist properties in this setting [28, 29]. This is largely due to challenges in accepting approaches that can raise the type 1 error such as informative Bayesian priors when estimating vaccine efficacy. Regulators often would also expect to see results from a standalone trial, without the influence of prior information.

Additionally, other concerns were raised about Bayesian trial designs. Defining type I error in the Bayesian setting relies on simulations, for which the same standards should apply; however, what remains unclear are the situations in which compliance to these standards can be robustly demonstrated. There was also discussion about whether there is any benefit of using a Bayesian approach, given Bayesian approaches often need to be benchmarked against equivalent frequentist concepts (i.e., power) in the context of trial design. Finally, there may be issues with combining data from Phase II and Phase III trials given potential differences in manufacturing methods for the vaccine and/or definitions of endpoints considered in the trials.

While there was limited support for applying these approaches to RCTs used for licensure, there was discussion about whether statistical methods that integrate data from multiple sources could be applied to post-licensure studies or to support decision-making. When the focus is on estimating real-world effectiveness, these methods could provide a more timely and reliable assessment. This may be particularly valuable for quantifying real-world vaccine effectiveness against rarer but more clinically important outcomes, e.g., severe disease or death. For example, such methods could be used to evaluate the effectiveness of nirsevimab against hospitalization or intensive care unit admission due to RSV; furthermore, they could be used for safety evaluations of adverse events following receipt of maternal vaccines.

### *Sequential trial designs*

In sequential trial designs, vaccine efficacy is evaluated on an ongoing basis, and the trial is stopped when sufficient evidence to establish efficacy has been collected. Sequential trial designs can be a powerful tool to assess the efficacy and safety of an intervention during the trial's course. Such designs are particularly useful when there is a high degree of uncertainty in key determinants of the sample size, such as the incidence rate of the primary endpoint. However, conducting multiple interim analyses requires using a more stringent P-value, which can increase the overall duration and cost of the trial [27].

In sequential trials, there is a trade-off between the number of interim analyses and the final sample size. By limiting the number of interim analyses, the penalty for additional analyses can be minimized. Increasing the number of interim analyses from two to five may result in a significant increase in the penalty, but beyond a certain threshold, further analyses will not have a substantial impact on the required sample size [27]. Having a high frequency of evaluation of efficacy would be possible with certain analytical strategies. However, the utility of such an approach was questioned by participants. As noted by participants, trials with sequential designs with a limited number of interim evaluations are already in routine use.

*General considerations on the need for more efficient trial designs for RSV*

There was a general discussion as to whether having smaller, more efficient trials is desirable. Stopping the trial earlier might make it even harder to evaluate an intervention's efficacy in specific subgroups or geographic regions. This information could be crucial for advisory bodies to make recommendations for use post-licensure. Additionally, studies not powered for critical subgroup analyses or safety outcomes may fail to adequately assess a potential safety issue that could represent a true signal. For instance, the maternal RSVpreF vaccine has shown a higher (though not statistically significant) rate of preterm birth in the vaccine arm, raising concerns about a potential true safety signal. In the context of regulatory interactions and committee meetings for RSV maternal vaccines, it has often been stated that the bare minimum sample size should not be the goal. Rather, larger trials are needed to provide comprehensive data across various settings and populations. Ideally, each country would want to understand whether the intervention works (and is safe) in their specific context.

For RSV in infants, and young children in particular, reducing the required sample size may not be a first priority, since RSV disease is relatively common (although severe outcomes are much less common). RSV is typically a highly seasonal pathogen, with epidemics occurring during the winter months in most locations, though the seasonal patterns can vary between temperate and tropical regions. Although sequential trials with evaluations occurring after each season could be valuable, correctly anticipating the RSV season at each site and enrolling participants prior to the start of the season is much more critical.

## EXTRAPOLATING CLINICAL TRIAL RESULTS ACROSS SETTINGS

The third session focused on using meta-regression models to extrapolate clinical trial results across settings, including both high-income countries (HICs) and low- and middle-income countries (LMICs). Vaccine trials, including those for RSV vaccines, are often conducted across multiple sites, representing diverse settings. In deciding on study sites, researchers must balance the need for geographic representation with practical considerations of study logistics, cost, and participant recruitment. Since trials are typically not powered to provide subgroup- or country-specific results, meta-regression models could offer a framework for comparing results between sites and obtaining more robust, context-specific estimates of efficacy.

During the meeting, we discussed results from meta-regression models applied to data from real-world clinical trials and case-control studies that evaluated rotavirus vaccines, and we discussed extensions and future directions in the context of RSV.

### *Detecting heterogeneity in vaccine efficacy across settings*

Clinical trials are usually not designed to detect heterogeneity in vaccine efficacy estimates across different settings. When heterogeneity is observed, it may be due to chance or differences in the interpretation of case definitions and criteria for hospitalization (when using hospitalized disease as an outcome) among study participants and clinicians in different settings.

Heterogeneity in vaccine efficacy estimates may also reflect true differences in vaccine performance across countries or settings. The efficacy of a vaccine might differ between HIC and LMIC settings due to variations in natural exposure and other factors such as malnutrition and the presence of co-infections. For example, the efficacy of rotavirus vaccines has generally been found to be substantially higher in HICs than in LMICs [30]. Conversely, in a post-hoc analysis of the Prepare trial of a maternal RSV vaccine candidate [21], vaccine efficacy was higher in LMIC sites than in HIC sites, although the difference in efficacy was not statistically significant.

During the meeting, a meta-regression framework was presented in which data on widely available predictors (e.g. under-5 mortality rate, WHO region, diarrhea prevalence, gross domestic product per capita) were used to explain variation in estimates of rotavirus vaccine efficacy (and effectiveness) across countries. This framework was then used to extrapolate estimates of vaccine performance to countries without efficacy or effectiveness data. Local decision-makers may find this modeling approach useful, as it is similar to burden modeling used to predict country-specific incidence when estimates are unavailable. However, it requires that there are

sufficient efficacy or effectiveness data available from a variety of countries and that widely available predictors of heterogeneity in vaccine efficacy can be identified.

### *Optimal trial site distribution using meta-regression models*

Pharmaceutical companies prioritize product licensure and often do not focus on having sufficient enrollment in individual sites or countries, particularly LMICs, to robustly estimate site- or region-specific efficacy. However, such information is critical to informing policy, as national and regional bodies that make recommendations about vaccines often desire clinical trial data for their specific context. From the perspective of advisory groups such as the WHO Strategic Advisory Group of Experts on Immunisation (SAGE), there is often criticism about limited trial data on vaccine efficacy coming from LMICs [30], and they may request additional trials and/or an impact study to assess effectiveness before making wider recommendations for vaccine use [31, 32].

Meta-regression models can be utilized to identify data gaps and inform optimal data collection strategies and trial site distribution. They can be helpful in finding the best trade-off between a well-distributed global representation of participants and maintaining sufficient statistical power at each study site to ensure robust local estimates. It is important to understand the amount of data required to determine how and if vaccine efficacy varies across settings and to enable robust extrapolation across settings.

### *Using a minimum threshold to determine vaccine efficacy*

Instead of aiming for precise point estimates of vaccine efficacy for individual countries or regions, it may be more productive to assess whether a minimum threshold of protection is met within that region. In other words, the focus would be on having the lower 95% confidence bound exceed a certain value of vaccine efficacy, which would depend on the specific product under consideration. Determining the minimum efficacy threshold requires careful consideration of various factors, including cost-effectiveness, and examples of how this approach has been used in practice would be useful. Incorporating uncertainty ranges in vaccine efficacy estimates in value of information analyses can identify whether these uncertainties should drive decision-making.

*Overall opinion on heterogeneity in vaccine performance*

Overall, the participants agreed that factors such as malnutrition, HIV, or malaria could lead to heterogeneity in the efficacy of vaccines against RSV, particularly for maternal vaccines. However, heterogeneity in vaccine performance will generally be assessed post-licensure rather than in clinical trials, and this type of meta-analytic approach can be useful for understanding predictors of heterogeneity and extrapolating vaccine efficacy and effectiveness estimates across countries.

## CONCLUSIONS

The recent meeting on the design and use of clinical trials for RSV vaccines and other preventive interventions covered crucial topics that require urgent attention given the high number of products in the clinical development pipeline. The meeting highlighted a lack of alignment between what is necessary for licensure and what is required post-licensure for informing vaccine deployment decisions, particularly in LMICs. One of the key debates was around whether we should prioritize increased efficiency to achieve licensure more quickly and inexpensively, or aim for more robust and larger trials to provide critical information at the country or regional level and to provide accurate information on safety, especially when concerning rare events.

The approaches covered in the workshop could be particularly valuable in the context of post-licensure studies. These methods are especially useful when the objective is to validate the effectiveness of vaccines within new or specific subgroup populations. It is our hope that this workshop will serve as a starting point for further discussions and meetings that will bring together regulatory and advisory bodies, including those in LMICs, to address these critical issues.

## NOTES

*Disclaimer*

The views expressed in this article are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the agencies or organizations with which the authors are affiliated.

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# Chapter 9

## Incorporating Data from Multiple Endpoints in the Analysis of Clinical Trials: Example from RSV Vaccines

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## ABSTRACT

**Background**:

To meet regulatory approval, interventions must demonstrate efficacy against a primary outcome in randomized clinical trials. However, when there are multiple clinically relevant outcomes, selecting a single primary outcome is challenging. Incorporating data from multiple outcomes may increase statistical power in clinical trials. We examined methods for analyzing data on multiple endpoints, inspired by real-world trials of interventions against respiratory syncytial virus (RSV).

**Methods**:

We developed a novel permutation test that represents a weighted average of individual outcome test statistics (*wavP*) to evaluate intervention efficacy in a multiple endpoint analysis. We compared the power and type I error rate of this approach to two methods: the Bonferroni correction (*bonfT*) and the *minP* permutation test. We evaluated the different approaches using simulated data from three hypothetical trials in which the intervention efficacy, the correlation, and the incidence of the outcomes varied, as well as data from a real-world RSV clinical trial.

**Results**:

When the vaccine efficacy against different outcomes was similar, *wavP* yielded higher power than *bonfT* and *minP;* in some scenarios the improvement in power was substantial. In settings where vaccine efficacy was notably larger against one endpoint compared to the others, all three methods had similar power. We developed an R package, *PERMEATE,* to guide selection of the most appropriate method for analyzing multiple endpoints in clinical trials.

**Conclusions**:

Analyzing multiple endpoints using a weighted permutation method can increase power while controlling the type I error rate compared to established methods under conditions mirroring real-world RSV clinical trials.

## INTRODUCTION

To receive licensure from a regulatory body like the Food and Drug Administration (FDA), investigators need to demonstrate that new vaccines are efficacious in a Phase 3 randomized controlled trial. Trials need to be designed to have the greatest probability of success in the most efficient way for two main reasons. First, Phase 3 trials have become increasingly complex and costly in recent years [1]. Secondly, by definition, clinical trials expose participants to experimental vaccines that have potential benefits but also have potential safety issues.

Phase 3 trials typically must specify one or more primary endpoints, and the ultimate success or failure of the trial hinges on the ability to demonstrate a significant reduction in the occurrence of the primary endpoint among those receiving the intervention. The primary endpoint needs to be carefully selected based on clinical relevance, ability of the vaccine to prevent the outcome, and the incidence rate in the study population. When there are multiple possible clinically relevant outcomes that are related, it is particularly difficult to determine which endpoint should be designated as "primary". Incorporating data from multiple endpoints (or 'outcomes') in evaluations of vaccine efficacy could provide a more complete understanding of the effects of the intervention and increase the statistical power for detecting a true effect [2-4]. In general, there are two main advantages to including multiple endpoints in the primary analysis: (i) to increase the power of statistical tests and thereby reduce the required sample size of the trial, and (ii) to describe treatment effects more comprehensively, specifically for diseases where a single outcome measure does not fully represent the treatment effect [4, 5]. Therefore, analyzing multiple endpoints instead of a single endpoint could potentially avert wasted research effort.

As an example of the pitfalls of selecting a single clinical endpoint, a maternal RSV vaccine failed to achieve licensure in 2020 because it did not show efficacy against the primary endpoint of medically significant RSV lower respiratory tract infection (LRTI) [6]. The trial only slightly missed the threshold for statistical significance [6]. However, the vaccine did show efficacy against related secondary endpoints, including hospitalization with RSV LRTI. If the analysis plan of the maternal RSV vaccine had incorporated data from multiple endpoints, it may have been reasonable to conclude that the vaccine was efficacious [6]. However, the rigidity of the regulatory process led to this potentially useful vaccine being largely abandoned.

Choosing the best approach to analyze data on multiple endpoints in clinical trials depends on the objectives and design of the trial, as well as the characteristics of the endpoints themselves [7]. When analyzing multiple endpoints, it is important to control the type I error rate (i.e., the probability of falsely rejecting the null hypothesis of no vaccine effect when it is true)[8]. The most common approach for doing so is to analyze

each endpoint independently and adjust the corresponding P-values using the Bonferroni correction [8-10]. However, this approach does not account for the correlation between endpoints, which typically leads to overly conservative P-values and lower power compared to other approaches [2, 11-13]. For instance, most hospitalizations with RSV LRTI are considered medically significant, but not all medically significant RSV LRTI will be hospitalized. Resampling methods, such as permutation approaches, could provide more power when there is correlation between endpoints, while at the same time maintaining control of the overall type I error rate [2, 5, 7, 10, 14]. However, despite the increasing use of multiple endpoints in randomized clinical trials, guidance is still limited on how to analyze and interpret the resulting data [2, 15]. Finally, composite endpoints offer an alternative approach to incorporating data from multiple endpoints [16]. By considering a subject to have the composite endpoint if they experience outcome A OR outcome B, the study's power is increased. However, this approach does not differentially weight outcomes based on different criteria and might be suboptimal in settings where the vaccine efficacy differs substantially across different components of the composite.

Here, we propose and evaluate a novel approach for analyzing data on multiple endpoints where we derive a permutation-based test statistic that represents a weighted average of individual outcome test statistics. We compare the power and type I error rate of this approach with two established methods using simulated data that have different vaccine effect sizes, incidence estimates, and levels of correlation among the endpoints, mirroring real-world RSV clinical trials. Additionally, we analyze multiple endpoint data from a real-world clinical trial using these methods and compare the findings. Our aim is to provide practical recommendations on the preferred method based on the specific setting, along with tools for implementing the approaches we describe.

## METHODS

We compare the performance of three methods for analyzing multiple endpoints across different simulated scenarios. The first method, the Bonferroni correction (*bonfT*), is one of the most common approaches for analyzing multiple outcomes due to its simplicity in application. The second and third methods are permutation tests, which allow us to flexibly combine the test statistics from multiple outcomes into a single metric for making statistical inference. For all methods, we used the risk ratio (RR) as our estimand for the individual endpoints, which is directly connected to vaccine efficacy (i.e., vaccine efficacy = 1 - RR).

### *Bonferroni correction*

A common approach for analyzing data on multiple endpoints is to first analyze each outcome independently [8-10]. In this setting, we use a two-sample test of proportions comparing the event rate in the intervention and control arms to obtain P-values for each endpoint. Similar to the Prepare trial [6], we use a one-sided hypothesis test for calculating the type I error rate.

To correct for the multiple hypothesis tests being performed, *bonfT* divides the original statistical significance level (i.e., $\alpha$ level, here set to 0.05) by the total number of outcomes (i.e., $M$). The method considers a treatment effect to be statistically significant if any of the endpoint-specific P-values are less than $\alpha/M$.

### *Permutation tests*

Permutation methods estimate the sampling distribution of a selected test statistic when the null hypothesis is true by repeatedly "shuffling" the treatment status of each individual in (i.e., control or intervention group) [17]. The test statistic is then calculated as described below. The actual test statistic from the observed data (i.e., with correct group labels) is compared to this null distribution; a P-value is then calculated based on the quantile where the actual test statistic falls on the null distribution (Figure S1).

### *Test statistics*

For each permuted dataset, the minimum P-value approach (*minP*) first computes a P-value for each endpoint based on the individually analyzed RRs and then selects the smallest P-value as its test statistic [18]. Similar to *bonfT*, we used a one-sided two-sample test of proportions to obtain the P-values for each endpoint. The test statistic

for a single permuted dataset is given as $minP = \min\left(p_{value\,j}\right)$ for outcome $j \in \{1, \ldots, M\}$.

Alternatively, we developed a novel approach where we pooled the RRs from each outcome together using a weighted average (Weighted Average Permutation test, *wavP*). Specifically, we calculated the RR for each outcome and created a summary test statistic by multiplying each RR (on the log scale) by a factor proportional to the inverse of its variance, such that the test statistic is given as:

$$wavP = \sum_{j=1}^{M} \log\left(RR_j\right) w_j$$

where $w_j = \frac{1/\mathrm{Var}(\log(RR_j))}{\sum_{j=1}^{m} 1/\mathrm{Var}(\log(RR_j))}$; $\mathrm{Var}(\log(RR_j)) = \frac{1}{Y_{t,j}} + \frac{1}{Y_{c,j}} - \left(\frac{1}{X_t} + \frac{1}{X_c}\right)$; $Y_{t,j}$ and $Y_{c,j}$ are the number of cases of endpoint *j* in the treatment and control arms, respectively; $X_t$ and $X_c$ are the number of individuals in the treatment and control arms, respectively.

For each dataset, simulated or permuted, with zero occurrences in the treatment or control arms, we included a continuity correction factor for sparse counts [19]. We modified the definition of the RR and variance as:

$$RR_j = \frac{(Y_{t,j} + k)/(X_t + 2k)}{(Y_{c,j} + k)/(X_c + 2k)}$$

$$\mathrm{Var}(\log(RR_j)) = \frac{1}{Y_{t,j} + k} + \frac{1}{Y_{c,j} + k} - \left(\frac{1}{X_t + 2k} + \frac{1}{X_c + 2k}\right)$$

where $Y_{t,j}$ and $Y_{c,j}$ are the number of cases of endpoint *j* in the treatment and control arms, respectively; $X_t$ and $X_c$ are the number of individuals in the treatment and control arms, respectively; and $k$=0.5 is the continuity correction factor [19].

We tested additional approaches by varying how the test statistic was constructed; in one case, we used the product of RRs as our test statistic; in another case, we used a summary test statistic where each RR was multiplied by the weighted average of the outcomes in the control group. However, these methods did not perform as well as *wavP* (Tables S1 and S2 in the supplementary file).

### *Creating the null distribution*

We randomly shuffled the treatment and control labels for the individuals $N$=999 times to obtain a distribution of the permuted test statistics for both $minP$ and $wavP$. In parallel, we computed the same test statistics for the observed data, deriving $minP_{obs}$ and $wavP_{obs}$.

The permutation P-value is defined as the proportion of permuted datasets that have a test statistic equal to or more extreme than that of the observed data. This corresponds to a one-tailed test (Figure S1 in the supplementary file). Specifically, the P-values are defined as $p_{minP} = \sum_{i=1}^{N} 1(minP_i \leq minP_{obs})/N$ and $p_{wavP} = \sum_{i=1}^{N} 1(wavP_i \leq wavP_{obs})/N$ for the two test statistics, respectively. The statistical significance level was set at 0.05 for these tests (i.e., $\alpha$ =0.05).

*Data simulation*

To test the performance of the three different methods for analyzing multiple endpoints, we performed a study in which 10,000 different datasets were simulated and analyzed with each method. We considered several scenarios in which we varied the number of individuals in the control and treatment groups, the expected incidence of each outcome in the treatment group, and the effect of the intervention against each outcome (as quantified by the risk ratio). For each scenario, we varied the correlation among outcomes between 0.01 and 0.8.

Individual-level binary outcomes for each endpoint (i.e. whether or not the outcome occurred during the follow-up period of the trial) were independently generated using the R package *bindata* [20], which first simulates a correlated vector of continuous responses based on treatment status (e.g., vaccination status) and then dichotomizes those responses as above/below zero to create the binary outcome. For example, when considering two endpoints, the vector of binary response for individual *k*, $Y_k^{\mathrm{T}} = (Y_{k1}, Y_{k2})$, was simulated as $Y_{kj} = 1(\beta_{0j} + \beta_{1j}\mathrm{z}_k + \epsilon_{kj} > 0), j = 1,2$, where $\mathrm{z}_k$ indicates whether participant *k* received the intervention ($\mathrm{z}_k$=1) or control ($\mathrm{z}_k$=0), $\beta_{0j}$ specifies the proportion of the control group that experiences the outcome of interest during the follow-up period, $\beta_{1j}$ is the intervention effect for outcome *j*, and $\epsilon_{kj}$ are correlated errors simulated from a multivariate Gaussian distribution, such as $\epsilon_k^{\mathrm{T}} = (\epsilon_{k1}, \epsilon_{k2}) \sim \mathrm{MVN}\left(\binom{0}{0}, \begin{pmatrix} 1 & \rho \\ \rho & 1 \end{pmatrix}\right)$, with $\rho$ representing the correlation coefficient. Correlation in the latent continuous variables results in correlation between the binary responses specific to an individual, regardless of treatment status. We selected $\beta_{0j}$ and $\beta_{1j}$ parameters so that the incidence in the control and treatment arms was $\lambda_{c,j}$ and $\lambda_{t,j}$, respectively (i.e., the proportion of participants in the control and treatment arms experiencing the outcome of interest). This model was extended to simulate three outcomes [2].

We assumed each person could develop the *M*=3 different outcomes, with the degree of correlation among the outcomes varied between 0.01 and 0.8, $\rho \in \{0.01, 0.2, 0.4, 0.6, 0.8\}$. Since the correlated binary outcomes are generated by thresholding a continuous distribution, the corresponding correlation among the

outcomes differed in comparison to the input correlation and varied depending on the simulation seed and the scenario (Table S3 in the supplementary file). They were generally lower overall but maintained the expected ordering. Also, our approach assumed all-or-nothing vaccine protection. After simulating the data, we computed the observed RR for each outcome as $RR_{obs,j} = \frac{Y_{t,j}/X_t}{Y_{c,j}/X_c}$, with $X_t$, $X_c$, $Y_{t,j}$ and $Y_{c,j}$ previously defined.

We simulated data based on three different scenarios, inspired by three published clinical trials of RSV prophylaxis and vaccines. The MAKI trial was a study of the effect of palivizumab, a monoclonal antibody used for RSV prophylaxis, for prevention of childhood wheeze (scenario A)[21]; the Melody trial was a phase 3 trial testing the effectiveness of extended half-life RSV prophylaxis, nirsevimab, against lower respiratory disease in infants (scenario B) [22]; and the Prepare trial was a phase 3 trial of a maternal vaccine, RSV-F, for the prevention of RSV-associated lower respiratory disease in infants (scenario C) [6].

In the "MAKI-type" simulated data, we assumed a similar number of participants in each arm, with $X_t = X_c = 200$, high incidence of the three outcomes in the control arm, i.e. the proportion of participants in the control arm experiencing the outcome of interest ($\lambda_{cj}$~ 0.12-0.22), similar effect sizes of the intervention against the three outcomes ($RR_j$~ 60-70%), and expected number of cases equal to 20-50 for the three outcomes [21]. The "Melody-type" data had a 2:1 ratio between individuals randomized to the treatment and control arms, with $X_t = 996$ and $X_c = 496$, low incidence of the three outcomes ($\lambda_{cj}$~ 0.02-0.05), corresponding to 10-50 expected cases, and heterogeneous effect sizes ($RR_j$~ 25-60%) [22]. Finally, the "Prepare-type" data had more participants than the other two trials, with $X_t = 2{,}765$ and $X_c = 1{,}430$, low incidence of the three outcomes ($\lambda_{cj}$ ~ 0.01-0.04), corresponding to 10-40 expected cases, and comparable effect sizes against the various outcomes ($RR_j$ ~ 50-60%); in the latter setting, the intervention had the strongest effect size against the outcome with the lowest incidence [6]. Details of the different simulated scenarios are summarized in Table 1.

### *Power and type I error*

We computed the power of each method as the proportion of the 10,000 simulations for which a significant effect was detected, as previously defined for each method. Similarly, we computed the type I error by setting $RR_j$=1 for each outcome and then calculating the proportion of datasets for which we incorrectly found a significant effect.

**Table 1.** Data simulation. Three scenarios were tested with different sample size, effect size, and incidence. For all scenarios, the correlation among the outcomes was varied between $\rho$=0.01 and $\rho$=0.80.

| Scenario | Sample size number of participants in the control ($X_c$) and treatment ($X_t$) groups | Incidence of each outcome in the control arm ($\lambda_{c,j}$) | Intervention effect size risk ratio against outcome $j$ ($RR_j$) | Reference clinical trial | Example outcomes for RSV trials |
|---|---|---|---|---|---|
| Scenario A) High and comparable effect size and incidence | $X_c = X_t$ =200 | $\lambda_{c,1}$=0.22, $\lambda_{c,2}$=0.20, $\lambda_{c,3}$=0.12 | $RR_1$=0.60, $RR_2$=0.60, $RR_3$=0.70 | MAKI trial [21] | Childhood asthma, wheeze, and use of asthma medications |
| Scenario B) Low incidence and heterogeneous effect size | $X_c$ =496, $X_t$ =994 | $\lambda_{c,1}$=0.05, $\lambda_{c,2}$=0.02, $\lambda_{c,3}$=0.03 | $RR_1$=0.25, $RR_2$=0.40, $RR_3$=0.60 | Melody trial [22] | Medically attended RSV LRTI, hospitalization for RSV LRTI, and hospitalization for any RSV in children |
| Scenario C) Low incidence and high effect size | $X_c$ =1,430, $X_t$ =2,765 | $\lambda_{c,1}$=0.02, $\lambda_{c,2}$=0.04, $\lambda_{c,3}$=0.01 | $RR_1$=0.60, $RR_2$=0.55, $RR_3$=0.50 | Prepare trial [6] | Medically significant RSV LRTI, RSV LRTI with severe hypoxemia, and hospitalization for RSV LRTI |

*Real-world data: RSV prevention and asthma in healthy preterm infants in the Maki trial case study*

We also tested the different analytical approaches using real-world clinical trial data from the MAKI trial. MAKI was a double-blind, randomized, placebo-controlled trial involving 429 infants born at 32-35 weeks of gestation recruited during their first year of life between 2008-2010 [21, 23]. Participants were randomized to receive palivizumab prophylaxis or placebo. The trial reported a causal relationship between prevention of RSV infection during infancy and reduced frequency of subsequent wheeze [23]. Here, we analyzed the data coming from the single assessor-blind follow-up of trial participants at the age of six years [21]. Primary endpoints included current asthma, defined as parent-reported wheeze in the past 12 months or parent-reported use of asthma medications in the past 12 months, or both. The second primary endpoint was forced expiratory volume in 0.50 seconds, assessed by spirometry. In our analysis, we explored three different scenarios focusing on the parent-reported outcomes only. The scenarios differed based on the number and type of outcomes and were: 1) three

outcomes with high incidence: current asthma, wheeze in the past 12 months, and use of asthma medications in the past 12 months; 2) four outcomes: current asthma, wheeze in the past 12 months, use of asthma medications in the past 12 months, and nocturnal cough; 3) four outcomes: current asthma, wheeze in the past 12 months, use of asthma medications in the past 12 months, and use of oral corticosteroids.

### *R package PERMEATE*

We developed an R package, *PERMEATE* (PERmutation basEd ANalysis of mulTiple Endpoints), that returns the power and type I error rate for all presented methods based on user-defined expected effect sizes, incidence, and correlation among the outcomes. Additional documentation can be found [24].

## RESULTS

### *Data simulation results*

Scenario A) MAKI-type data: high and comparable effect size and incidence

When incidence was high, and the effect sizes were comparable for all the outcomes (similar to the Maki trial), $wavP$ strongly outperformed both *bonfT* and *minP* for correlation values ranging from $\rho$=0.01 to $\rho$=0.60. When there was high correlation between the outcomes (i.e., $\rho$=0.80), $wavP$ was still superior, though the difference was reduced compared with the *minP* method (**Figure 1A** and Table S4 in the supplementary file). The *minP* approach was superior compared to the *bonfT* across all levels of correlations. The type I error fluctuated around 5% for $wavP$, while it was consistently under 5% for *bonfT* and $minP$ for all correlation values (**Figure 1B** and Table S4 in the supplementary file).

Scenario B) Melody-type data: low incidence and heterogeneous effect size

When the incidence was low, and the effect size was heterogeneous among the outcomes (similar to the Melody trial), $wavP$ exhibited similar power results compared to *minP* and *bonfT* for correlation values ranging from $\rho$=0.01 to $\rho$=0.60. Instead, in a high correlation setting ($\rho$=0.80), the power from $wavP$ was slightly lower than that from *minP* and *bonfT,* but power was still >95% for all methods (**Figure 2A** and Table S4 in the supplementary file). The *minP* approach showed modest power gains compared to *bonfT* in all settings. The type I error was around 5% for $wavP$ and *minP* but was below the 5% threshold for *bonfT* (**Figure 2B** and Table S4 in the supplementary file).

Scenario C) Prepare-type data: low incidence and high effect size

Finally, in a setting where incidence was low and the rarest outcome had the strongest effect size (similar to the Prepare trial), $wavP$ outperformed both $minP$ and *bonfT* for all correlation values, with a small loss of power as the correlation among the outcomes increased (**Figure 3A** and Table S4 in the supplementary file). The $minP$ and *bonfT* approaches showed similar performances, with *minP* slightly superior for all correlation values. The type I error for $wavP$ fluctuated around 5%, while it was below the threshold for both $minP$ and *bonfT* (**Figure 3B** and Table S4 in the supplementary file).

For all three simulated scenarios, we also computed the power and type I error when testing each outcome independently, as it would be in a trial with a single endpoint (*Outcome1*, *Outcome2*, and *Outcome3* for each scenario). In general, the outcome with the highest incidence had the highest power when testing the outcomes independently (**Figures 4** to **6**). When comparing the independent results to a multiple endpoint analysis, *wavP* had greater power or similar power to the best performing individual outcome. *wavP* outperformed all approaches in scenarios A and C (**Figures 4** and **6**). In scenario B, the power was comparable for *minP*, *bonfT*, *wavP*, and when looking at *Outcome2* alone, with a small loss of power for *wavP* when correlation was high among the outcomes (**Figure 5A**).

*Analyses of Real-world data*

The MAKI trial case study: RSV prevention and asthma in healthy preterm infants

When we analyzed data on three outcomes from the MAKI trial, we found that all the methods returned significant P-values ($p<0.05$), with *wavP* showing the smallest P-value (p=0.0070). When adding an outcome with high incidence but almost no effect size (i.e., nocturnal cough) to the previous scenario, all methods again had significant P-values. Both *wavP* and *minP* had a higher P-value compared to the previous case (p=0.0360 and p=0.0290 respectively). Similarly, when adding a rare outcome with high effect size (i.e., use of oral corticosteroids), all methods had P-values under the 0.05 threshold, with *wavP* showing the lowest P-value (p=0.0080). In summary, all methods performed similarly in an analysis of this specific real-world dataset. Results are displayed in Table 2.

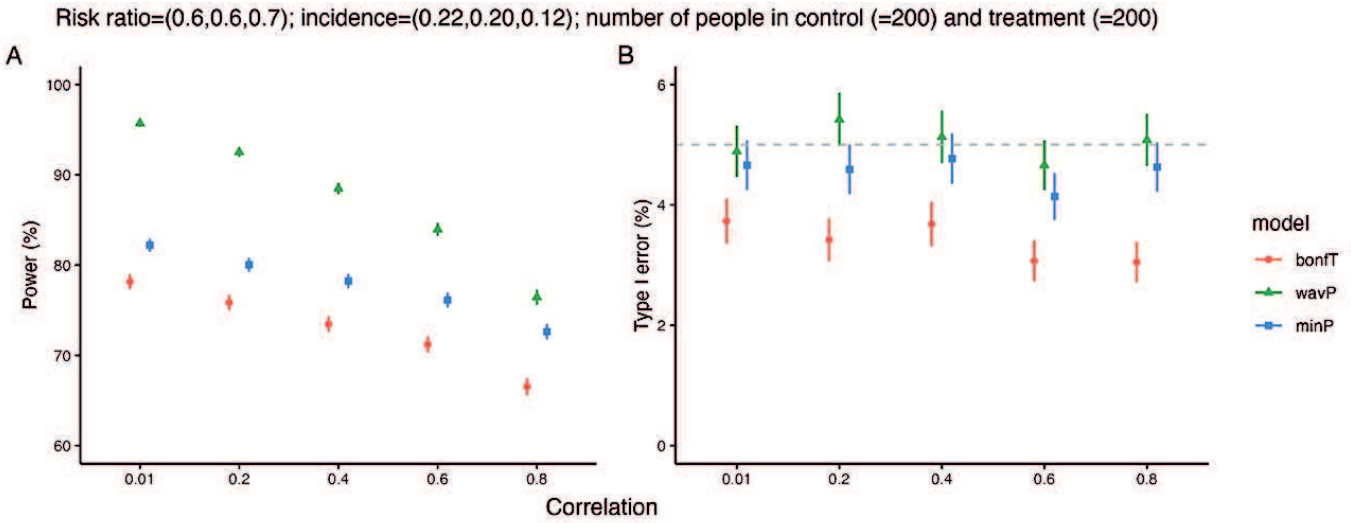



**Figure 1**: Data simulation of scenario A) MAKI-type data. Power (panel A) and type I error (panel B) with varying degrees of correlation among the three outcomes with $X_c = X_t = 200$, $RR_1$=$RR_2$=0.60, $RR_3 = 0.70$, and $\lambda_{c,1}$=0.22, $\lambda_{c,2}$=0.20, $\lambda_{c,3}$=0.12. Error bars represent the 95% confidence intervals; *wavP* and *minP* are the permutation methods, *bonfT* is the Bonferroni approach; $RR_j$= risk ratio for outcome *j*; $\lambda_{c,j}$=incidence in the control group for outcome *j*; $X_c$ = number of individuals in the control group; $X_t$ = number of individuals in the treatment group

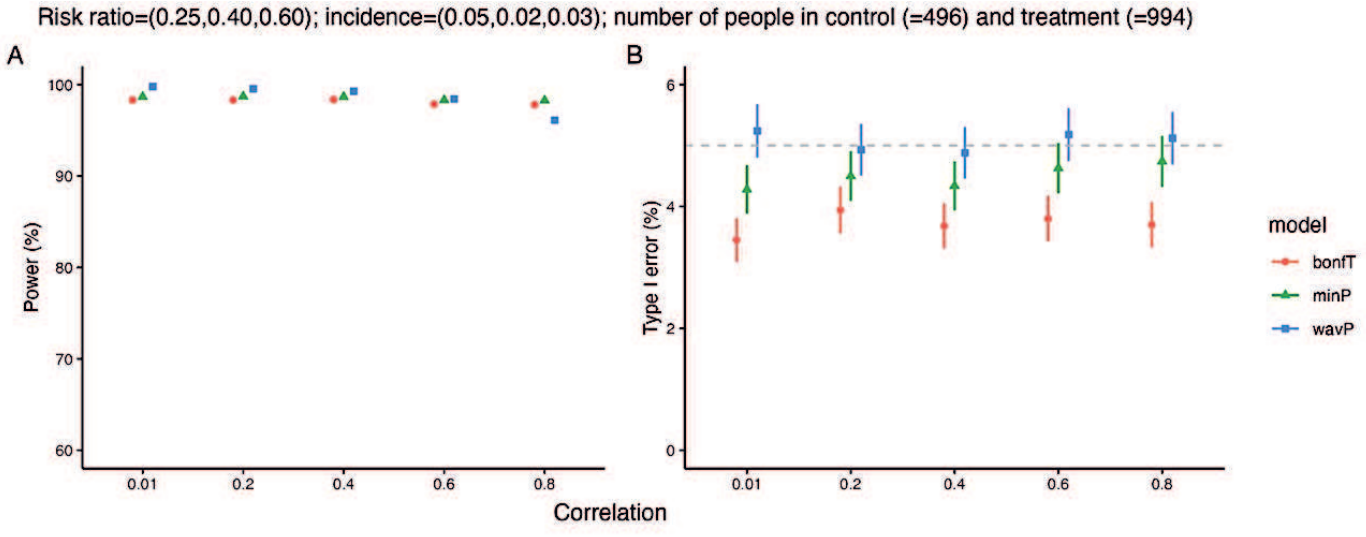



**Figure 2**: Data simulation of scenario B) Melody-type data. Power (panel A) and type I error (panel B) with varying degrees of correlation among the 3 outcomes with $X_c = 496, X_t = 994$, $RR_1$=0.25, $RR_2$ =0.40, $RR_3$ =0.60, and $\lambda_{c,1}$=0.05, $\lambda_{c,2}$ =0.02, $\lambda_{c,3}$ =0.03. Error bars represent the 95% confidence intervals; $wavP$ and *minP* are the permutation methods, *bonfT* is the Bonferroni approach; $RR_j$= risk ratio for outcome *j*; $\lambda_{c,j}$=incidence in the control group for outcome *j*; $X_c$ = number of individuals in the control group; $X_t$ = number of individuals in the treatment group.

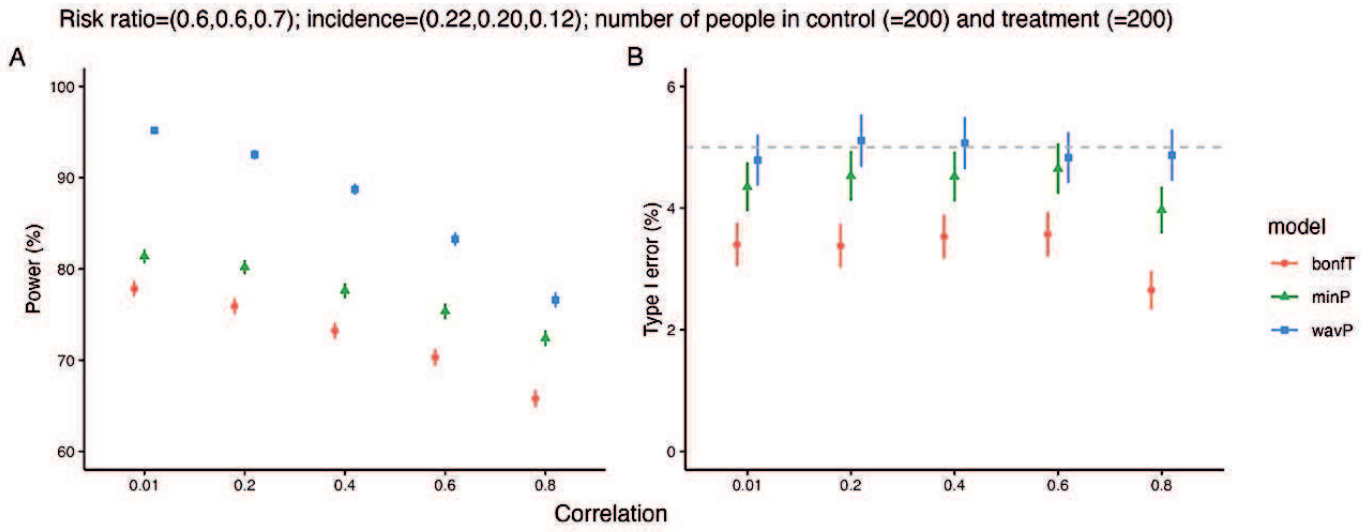



**Figure 3**: Data simulation of scenario C) Prepare-type data. Power (panel A) and type I error (panel B) with varying degrees of correlation among the 3 outcomes with $X_c = 1430, X_t = 2765$, $RR_1$=0.60, $RR_2$ =0.55, $RR_3$ =0.50, and $\lambda_{c,1}$=0.02, $\lambda_{c,2}$=0.04, $\lambda_{c,3}$=0.01. Error bars represent the 95% confidence intervals; $wavP$ and *minP* are the permutation methods, *bonfT* is the Bonferroni approach; $RR_j$= risk ratio for outcome *j*; $\lambda_{c,j}$=incidence in the control group for outcome *j*; $X_c$ = number of individuals in the control group; $X_t$ = number of individuals in the treatment group.

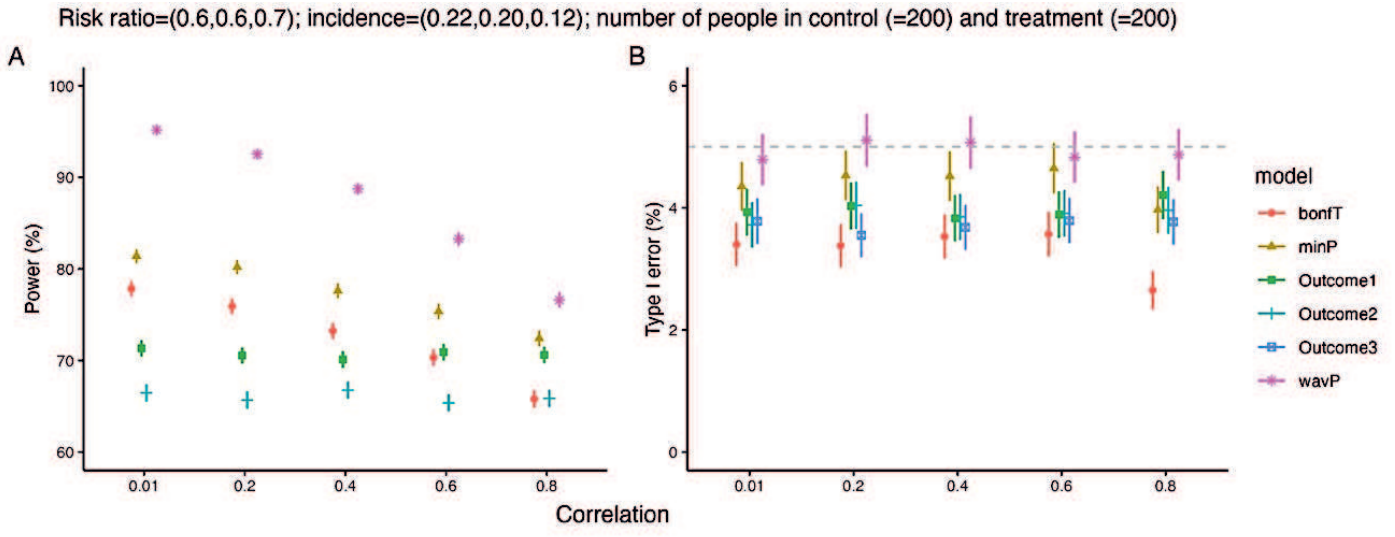



**Figure 4**: Data simulation of scenario A) MAKI-type data. Power (panel A) and type I error (panel B) with varying degrees of correlation among the three outcomes with $X_c = X_t = 200$, $RR_1$=0.60, $RR_2$ =0.60, $RR_3$ =0.70, and $\lambda_{c,1}$=0.22, $\lambda_{c,2}$ =0.20, $\lambda_{c,3}$ =0.12. Error bars represent the 95% confidence intervals; *wavP* and *minP* are the permutation methods, *bonfT* is the Bonferroni approach; *Outcome1*, *Outcome2*, and *Outcome3* represent the different outcomes analyzed individually; $RR_j$= risk ratio for outcome *j*; $\lambda_{c,j}$=incidence in the control group for outcome *j*; $X_c$ = number of individuals in the control group; $X_t$ = number of individuals in the treatment group.

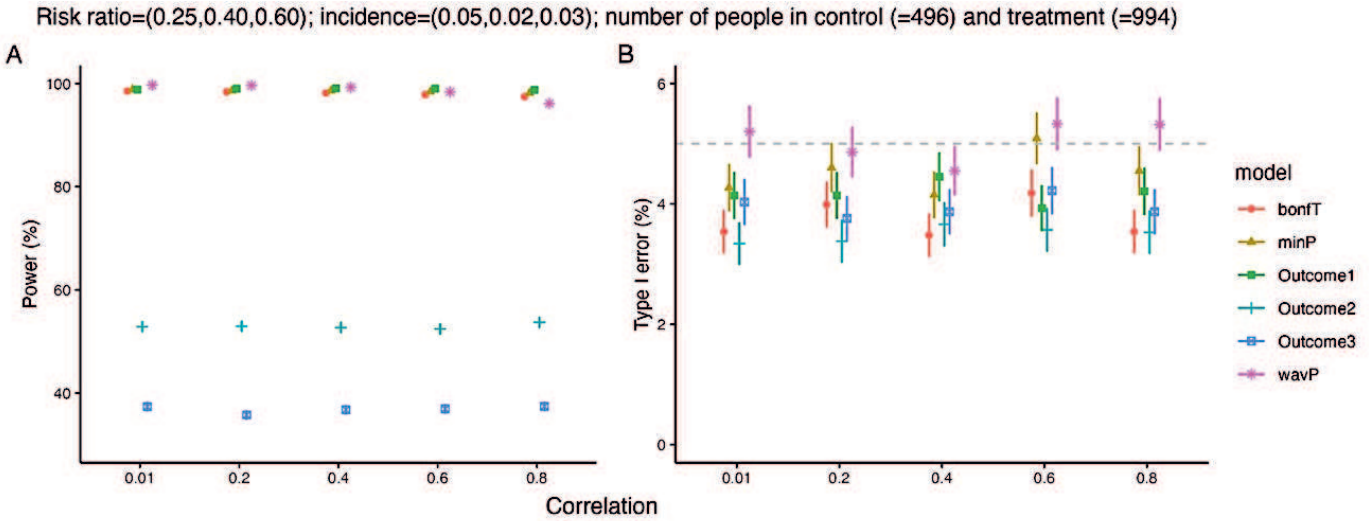



**Figure 5**: Data simulation of scenario B) Melody-type data. Power (panel A) and type I error (panel B) with varying degrees of correlation among the 3 outcomes with $X_c = 496, X_t = 994$, $RR_1$=0.25, $RR_2$ =0.40, $RR_3$ =0.60, and $\lambda_{c,1}$=0.05, $\lambda_{c,2}$ =0.02, $\lambda_{c,3}$ =0.03. Error bars represent the 95% confidence intervals; *wavP* and *minP* are the permutation methods, *bonfT* is the Bonferroni approach; *Outcome1*, *Outcome2*, and *Outcome3* represent the different outcomes analyzed individually; $RR_j$= risk ratio for outcome *j*; $\lambda_{c,j}$=incidence in the control group for outcome *j*; $X_c$ = number of individuals in the control group; $X_t$ = number of individuals in the treatment group.

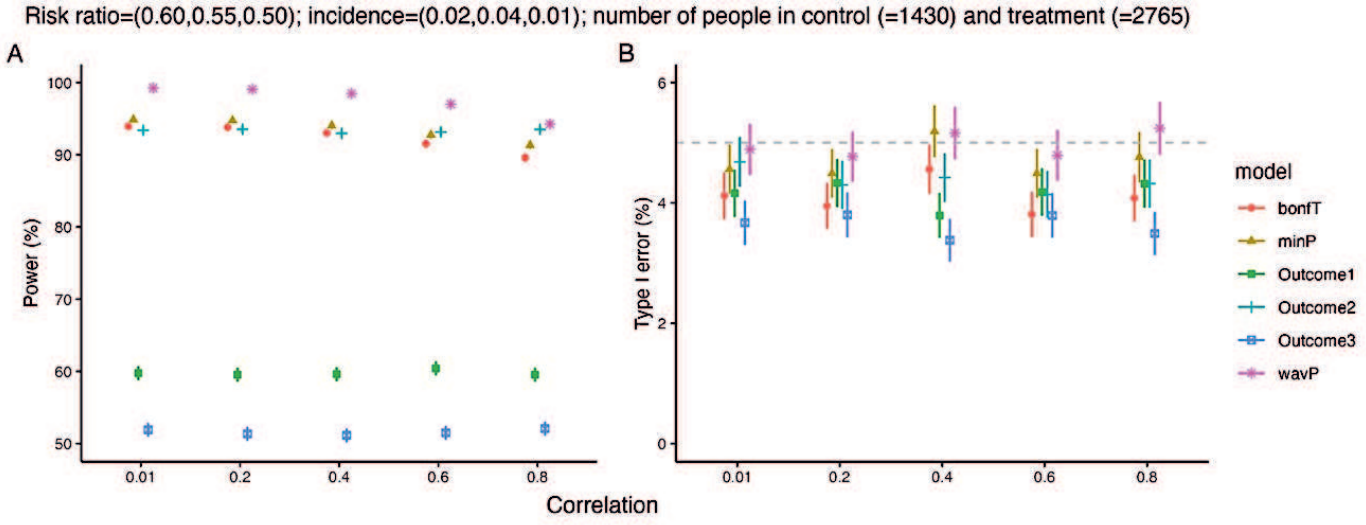



**Figure 6**: Data simulation of scenario C) Prepare-type data. Power (panel A) and type I error (panel B) with varying degrees of correlation among the 3 outcomes with $X_c = 1430, X_t = 2765$, $RR_1$=0.60, $RR_2$ =0.55, $RR_3$ =0.50, and $\lambda_{c,1}$=0.02, $\lambda_{c,2}$=0.04, $\lambda_{c,3}$=0.01. Error bars represent the 95% confidence intervals; *wavP* and *minP* are the permutation methods, *bonfT* is the Bonferroni approach; *Outcome1*, *Outcome2*, and *Outcome3* represent the different outcomes analyzed individually; $RR_j$= risk ratio for outcome *j*; $\lambda_{c,j}$=incidence in the control group for outcome *j*; $X_c$ = number of individuals in the control group; $X_t$ = number of individuals in the treatment group.

**Table 2.** Real-world data analysis of multiple endpoints from the MAKI trial. P-values for the three analytical approaches based on different multiple endpoints evaluated for the MAKI trial data. $\lambda_{c,j}$=incidence of outcome *j* in the control arm; $RR_j$= risk ratio for the effect of the intervention (palivizumab for RSV prophylaxis) on each outcome *j*, *j*=1,..,*M*. The individual outcomes were evaluated using a one-sided two-sample test of proportions [29, 30].

| Description | Selected endpoints (Incidence $\lambda$ and effect size RR) | Analytical approach | P-value |
|---|---|---|---|
| Three outcomes with comparable incidence and effect size | 1. Current asthma ($\lambda_{c,1}$=0.24, $RR_1$=0.59)<br>2. Wheeze ($\lambda_{c,2}$=0.20, $RR_2$=0.58)<br>3. Use of asthma medications ($\lambda_{c,3}$=0.13, $RR_3$=0.71) | $minP$ | 0.0120 |
| | | *wavP* | 0.0070 |
| | | *bonfT* | 0.0258 |
| Four outcomes with comparable incidence and effect size, and an additional outcome with high incidence and low effect size | 1. Current asthma ($\lambda_{c,1}$=0.24, $RR_1$=0.59)<br>2. Wheeze ($\lambda_{c,2}$=0.20, $RR_2$=0.58)<br>3. Use of asthma medications ($\lambda_{c,3}$=0.13, $RR_3$=0.71)<br>4. Nocturnal cough ($\lambda_{c,4}$=0.29, $RR_4$=0.91) | $minP$ | 0.0290 |
| | | *wavP* | 0.0360 |
| | | *bonfT* | 0.0344 |
| Four outcomes with comparable incidence and effect size, and an additional outcome with low incidence and high effect size | 1. Current asthma ($\lambda_{c,1}$=0.24, $RR_1$=0.59)<br>2. Wheeze ($\lambda_{c,2}$=0.20, $RR_2$=0.58)<br>3. Use of asthma medications ($\lambda_{c,3}$=0.13, $RR_3$=0.71)<br>4. Use of oral corticosteroids ($\lambda_{c,4}$=0.04, $RR_4$=0.49) | $minP$ | 0.0170 |
| | | *wavP* | 0.0080 |
| | | *bonfT* | 0.0344 |
| Individual p-values for the five different outcomes | 1. Current asthma ($\lambda_{c,1}$=0.24, $RR_1$=0.59) | | 0.0086 |
| | 2. Wheeze ($\lambda_{c,2}$=0.20, $RR_2$=0.58) | | 0.0162 |
| | 3. Use of asthma | | 0.1534 |

| | |
|---|---|
| medications ($\lambda_{c,3}$=0.13, $RR_3$=0.71) | |
| 4. Nocturnal cough ($\lambda_{c,4}$=0.29, $RR_4$=0.91) | 0.3334 |
| 5. Use of oral corticosteroids ($\lambda_{c,5}$=0.04, $RR_5$=0.49) | 0.1824 |

## DISCUSSION

In this study, we evaluated the performance of three different methods for estimating efficacy from vaccine trials that include multiple correlated endpoints. We focused on settings where no clear endpoint could be specified *a priori*, and the choice of the endpoint could determine the success or failure of the trial. We tested different methods for addressing multiple endpoints to provide practical recommendations in diverse scenarios based on the characteristics of the outcome and intervention, including incidence and effect size. In line with previous work [2, 15], we found that the use of multiple endpoints can lead to a better performance in terms of power, while keeping the type I error rate controlled, especially when there is no clear primary endpoint. Additionally, we proposed a novel weighted permutation approach, *wavP,* that pools the outcomes' test statistics together using a weighted average based on the inverse variances of the estimators. Using simulated and real-world data from clinical trials of RSV interventions, we show that our approach outperforms alternative methods in settings where the effect sizes among the outcomes are related. This finding holds significant relevance across all clinical trials featuring multiple endpoints nested within one another. It emphasizes that the overall value of the vaccine can be effectively demonstrated by synergistically combining these endpoints together.

Our analysis showed that methods such as *bonfT*, which treat each outcome independently, were robust in settings in which the intervention had a higher effect size against one outcome compared to the others (**Figure 2**); however, this method consistently exhibited lower power compared to other methods and was overly conservative with regards to type I error. Previous work demonstrated a superiority in power performances of methods that account for the correlation between outcomes, like permutation tests such as *minP* [2, 5, 14, 25]. We found that *minP* again performed well in settings where the intervention had a higher effect size against one outcome compared to the others. However, *minP* performed less optimally when the intervention had comparable effect size against the outcomes. For this reason, we developed a novel permutation approach, *wavP*, that pools the test statistics of the different outcomes together. This method uses an inverse-variance weighted average of the RRs (on the log scale) to combine the multiple endpoints such that outcomes with low uncertainty receive more weight. Our findings suggest that when the outcomes are related (**Figures 1, 3**), *wavP* outperforms the alternative methods. Finally, we tested the performances of each outcome individually. As expected, the outcome with the highest incidence consistently exhibited superior power performances; however, in most scenarios the power was lower compared to *wavP,* and comparable to *minP* and *bonfT*, further confirming the need to incorporate multiple endpoints in the analysis, particularly when the outcomes are related

(**Figures 4 to 6**). Finally, even if one of the outcomes analyzed independently had higher power compared to *wavP* for $\rho = 0.80$ (*Outcome 1* in **Figure 5A**), the difference was negligible, and opting for multiple endpoints mitigates the risk of selecting an incorrect outcome.

Given the large number of RSV vaccines that are in the clinical development pipeline, it is important to have efficient, well-designed studies that can evaluate these alternatives. This will continue to be important as additional vaccines move towards late-stage clinical trials and in trials that might compare the relative effectiveness of different preventive strategies more broadly.

Our work has several limitations. First, the simulated scenarios and approaches we explored are not exhaustive of all possible scenarios and of the large literature on multiple endpoint methods [13, 26]. We tested additional alternative approaches, but we found that performances were worse compared to our proposed method. Second, we chose to use *bonfT* as the benchmark because of its simplicity and broad applicability. However, the *bonfT* approach is known to be conservative, especially when there are a large number of positively correlated outcomes, resulting in a decrease of the type I error with a negative impact on the power; more advanced methods to correct for multiple testing have previously been proposed [7]. Lastly, the wavP approach might encounter challenges in settings where the treatment arm exhibits zero occurrences. While sparse data were not a significant concern in our simulation study, we addressed this issue by implementing a continuity correction factor in these specific settings [18]. It is worth noting that other approaches, such as Mantel-Haenszel estimators, might be more robust to small numbers of events [27, 28].

In our work, we focused on different approaches to deal with multiple and correlated outcomes. We developed a novel weighted permutation approach, and we compared the performances with other established methods using simulated and real-world data in the RSV field. We demonstrated how this permutation approach should be preferred when outcomes have shared effect size. As a result, we believe our approach is a robust and attractive method for analyzing clinical trial data from RSV products, and for vaccine-related clinical trials in general. We provide case-by-case guidance in our R package *PERMEATE* to help decide the most appropriate method for the design of clinical trials with multiple endpoints.

While this research does not delve into the technical, policy, and implementation aspects of using wavP in clinical trials, it is essential to give careful consideration to these factors. There remains a need to resolve questions concerning how the results of analyses utilizing multiple endpoints would be integrated into licensure and labeling decisions. For the purposes of developing a label for use, manufacturers and regulatory bodies need a clear indication of the vaccine's intended use. This may not

be feasible with an approach like wavP that integrates multiple outcomes, some of which might not reach statistical significance in the trial. Addressing these concerns is vital to ensure the meaningful application of this approach in clinical trial design and interpretation.

In conclusion, there is an acute need to design and implement more efficient randomized controlled trials for vaccines. This is needed to maximize the chances that an effective vaccine will receive licensure and will avoid wasting resources in underpowered or inefficient trials. Analyzing multiple endpoints using a weighted permutation method can increase power while controlling the type I error rate, particularly in settings where outcomes share similar effect sizes. Other novel trial designs that use Bayesian adaptive or sequential designs could also be considered. The adoption of these novel approaches could speed the introduction of needed products while maintaining rigorous standards of evaluation that are required in clinical trials.

## SUPPLEMENTARY MATERIALS



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# Chapter 10

## General Discussion



*Pre-licensure role of impact modelling*

Before clinical trial data are available, mathematical models are frequently used to simulate and predict population-level health outcomes in the context of novel vaccines and therapeutics, exploring a range of hypothetical uptake, efficacy, or scheduling scenarios [1,2]. Such models help forecast preventable mortality and estimate the benefits that a safe, effective, and affordable respiratory syncytial virus (RSV) intervention could provide [1,3].

Since 1998, a monoclonal antibody called palivizumab [2] has been the only licensed RSV immunization product, and its use was confined to a narrow group of high-risk infants in mostly high-income settings due to its high price and the requirement for monthly injections throughout the RSV season [4]. This profile made the intervention essentially out of reach for low- and middle-income countries (LMICs), where more than 97% of RSV-associated mortality occurs [5].

In the past three years, new RSV vaccines and monoclonal antibodies have become available in high-income countries. However, without financial support from Gavi, the Vaccine Alliance, these products remain out of reach for most LMICs [6]. Due to the high morbidity and mortality from RSV, Gavi committed in 2018 to supporting future RSV immunization products for eligible countries during the 2021–25 strategic funding period [7]. Gavi's Vaccine Investment Strategy (VIS) requires detailed assessments of disease burden, preventable mortality, cost-effectiveness, programmatic feasibility, and market readiness well before an intervention is licensed or prequalified [6,8]. Gavi identified the limited availability of country-specific and age-stratified burden data from LMICs as a major challenge for vaccine introduction [6,9].

**The main objective of this thesis was to inform cost-effectiveness modelers and decision-makers about the potential impact of maternal vaccination on RSV-related mortality in LMICs.** To achieve this, we refined an existing mathematical model and updated and validated its key inputs and underlying assumptions.

## EVIDENCE BEFORE THIS THESIS

*Burden*

RSV is estimated to be one of the leading causes of infant mortality and the primary cause of hospitalization in infants beyond the neonatal period (i.e., between 1 and 12 months of age)[10]. More than 95% of RSV-associated acute lower respiratory infection (RSV-ALRI) cases and approximately 97% of RSV-related childhood deaths occur in LMICs [5]. Globally, RSV accounts for approximately one in every 50 childhood deaths,

with an estimated 101,400 RSV-attributable deaths per year among children aged 0–60 months, including 45,700 in infants aged 0–6 months [5].

*Age at death distribution*

Because maternal vaccination and RSV monoclonal antibodies provide only temporary protection, **understanding the age distribution of RSV-related mortality in Gavi-eligible countries is essential for vaccine-impact modelling and cost-effectiveness analyses that inform Gavi's decisions** [6]. This is particularly important for infants younger than six months, who account for most RSV deaths and are the primary target population for these emerging interventions [11].

Perhaps the most influential global RSV burden estimates are those by Shi et al. (2017) [12], updated by Li et al. (2022) [5] together cited more than 4,400 times and referenced in Gavi's RSV Investment Case [7,13]. Previous RSV impact modelling and cost-effectiveness analyses, with a focus on LMICs, have relied on these estimates for their input parameters regarding age-specific incidence and mortality [3,14–17]. Importantly, these burden studies report age using broad three-month age bands, and the resulting mortality age distribution is not derived from directly observed data. Instead, mortality by age is largely inferred by combining age-specific incidence or hospitalization estimates with case fatality ratios. This approach overlooks the fact that the age profile of children hospitalized with RSV does not reflect the age profile of deaths: the youngest infants account for a disproportionately high share of severe and fatal outcomes, whereas older infants make up a larger proportion of non-fatal cases [6]. Recognizing this limitation, the WHO Strategic Advisory Group of Experts on Immunization (SAGE) underscored the major epidemiological gap in age-stratified data on RSV infection during the first months of life [18]. Additionally, despite the substantial burden of RSV morbidity in out-of-hospital cases, data from these populations remain extremely limited [9].

The RSV Global Online Mortality Database (RSV GOLD) was established in 2017 to characterize the clinical and sociodemographic profile of RSV-related pediatric deaths worldwide using original descriptions of individual children succumbing from RSV infection. Although this represented the largest hospital-based case series of RSV-related pediatric mortality at the time, only 117 fatal cases from low- and middle-income countries (LMICs) were included, which remains small relative to the estimated global burden [19]. Moreover, the analysis was limited to in-hospital deaths and did not capture community deaths, despite evidence[20] that the majority of global acute lower respiratory tract infection–related mortality occurs outside hospital settings.[19]

*Modelled vaccine impact on mortality*

In 2021, a systematic review of mathematical modelling of RSV in LMICs identified 3 studies that predicted the potential impact of maternal vaccination on RSV-related mortality [9]. As vaccine efficacy data were unavailable at the time, two studies assumed a fixed reduction in infections to explore the potential reduction in annual deaths [17,21]. The third study [22] developed a deterministic, mechanistic model to track how maternal RSV-specific antibody levels change from vaccination through birth and early infancy. This model suggested that vaccination at 30 weeks' gestation could prevent 29–48% of RSV-related in-hospital deaths globally. The analysis acknowledged several limitations, such as simplifying assumptions about antibody levels, optimistic coverage estimates, and limited data from low-income settings. The model parameterization is now outdated, as it was based on the Novavax maternal RSV vaccine, which failed to meet its primary efficacy endpoint in phase 3 trials and was not further developed [23], whereas a more promising vaccine (RSVpreF) has since emerged.

**In literature, little was known about the potential effectiveness of a maternal vaccine in preterm babies, who are more at risk of severe RSV disease**, although it was expected to be lower due to reduced transplacental antibody transport [22,24,25].

## SUMMARY OF KEY FINDINGS AND IMPLICATIONS FOR VACCINE IMPACT

*Conceptual framework*

**Figure 1** summarizes how the different components of this thesis contribute to vaccine impact estimation and, ultimately, to Gavi strategy decision-making.

Because protection conferred by maternal vaccination is time-limited, vaccine impact depends critically on when deaths occur in early life. **Part I** characterizes age-at-death distributions across infancy, which are critical for estimating the proportion of mortality that can be averted.

Building on this, **Part II** integrates the available epidemiological and immunological evidence and clinical trial inputs at the time into an impact modelling framework to estimate the potential impact of maternal vaccination. Starting from a theoretical maximum impact, progressively accounting for constraints related to antenatal care (ANC) timing and coverage, and vaccine acceptance yielded impact estimates that more closely approximate real-world outcomes.

**Part III** refines the immunological evidence base by characterizing antibody decay in early life, and the relation between placental health and maternal antibody transfer. These new insights were not incorporated in Part II, but are directly relevant for refining

assumptions on the duration and magnitude of protection and, therefore, for future impact modelling.

Finally, clinical trial evidence provides estimates of vaccine effectiveness in defined study populations, and **Part IV** reflects on how optimizing clinical trial design can generate outputs that are more readily usable in impact models, reducing reliance on extrapolation across settings and strengthening the link between trial evidence and impact estimation.

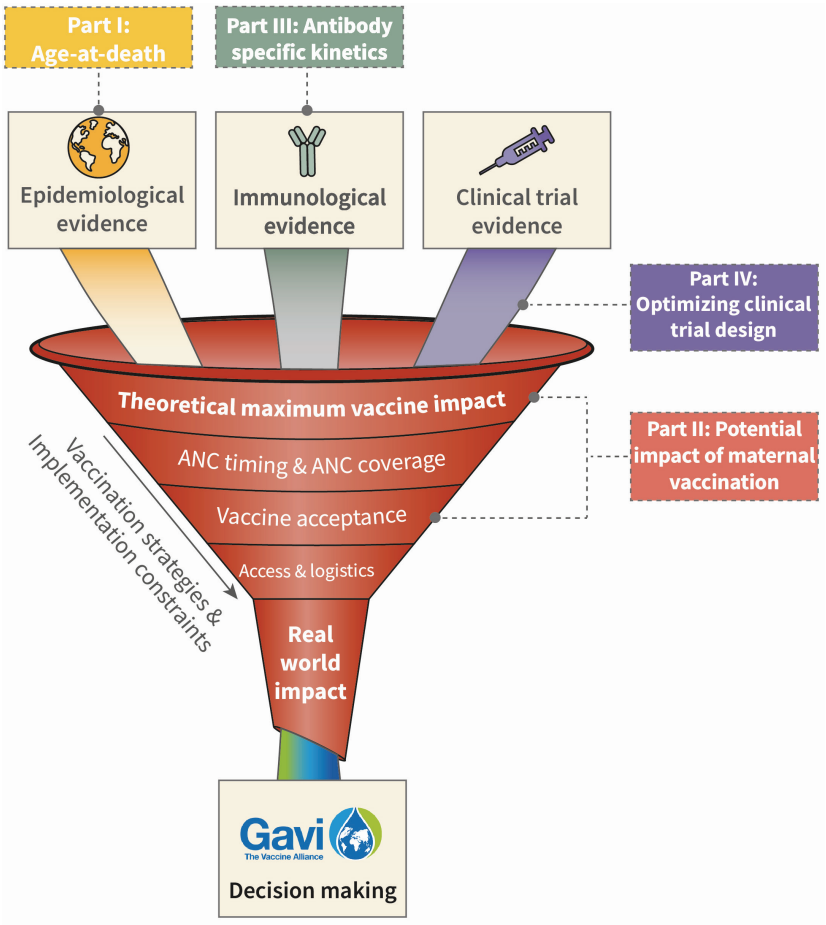



**Figure 1:** *Conceptual framework linking the thesis components to vaccine impact estimation and Gavi strategy decision making.*

## Part I: Age-at-death distribution

*Comparing community and in-hospital mortality*

In **Chapter 2** we studied 829 RSV-related deaths below 1 year of age from 38 developing countries. We aimed to describe global community pediatric deaths under 6 months and compare this group with in-hospital deaths in upper-middle-income countries (UMICs) and L(M)ICs.

**We observed that children dying in the community were significantly younger than those dying in hospital, with a median age at death of 1.5 months versus 2.4 months**, respectively, among infants who died before six months of age (**Chapter 2**). We therefore concluded that maternal vaccination or immunoprophylaxis against RSV will have a larger impact on RSV-related mortality in the community than in hospital. It is important to note, however, that 92% of the community deaths in this cohort originated from well-structured community mortality studies that used systematic post-mortem sampling and uniform laboratory methods, resulting in highly reliable data. For example, in the Zambia study [26], 2,286 infant deaths were sampled over a three-year period, representing 79% of all deaths in infants <6 months in the catchment area [27]. Among the in-hospital mortality cases in this cohort, only 14% originated from the same community-based mortality studies. The remaining cases were received from heterogeneous sources with both retrospective and prospective designs, including hospital record reviews in which testing was restricted to patients meeting site-specific protocols, as well as studies with variable inclusion criteria.

We addressed this potential bias in a sensitivity analysis. When the analysis was restricted to data from community-based mortality studies, the age difference between community and in-hospital deaths was smaller and no longer statistically significant. Moreover, in this sensitivity analysis, the proportion of neonates was similar among the in-hospital and community deaths. This suggested that **the observed difference in the main analysis was largely driven by differences in data collection methodology.**

*Impact of data collection methodology on age-at-death estimates*

In **Chapter 3**, we further examined how differences in data collection methods within the RSV GOLD mortality database influence estimates of age at death, focusing specifically on data from Gavi eligible countries. We applied two modelling approaches: one incorporating all registry and prospective data, and another restricted to the two prospective community mortality studies. Data collection methodology emerged as a significant predictor of age at death; **the prospective community mortality studies, largely based on systematic postmortem sampling,**

**yielded substantially lower age at death estimates than other registry data**. For example, the proportion of mortality below 6 months of age in children younger than 5 years, was 77% based on the prospective data model, compared with 59% in the model including all data sources (**Chapter 3**).

Several mechanisms may explain this discrepancy. Registry- and hospital-based surveillance systems are more likely to capture deaths occurring in better-resourced facilities or urban settings, introducing selection bias. In addition, RSV infection in very young infants may be under-recognized due to non-specific clinical presentation, whereas older children, particularly those with severe comorbidities, are more likely to be hospitalized, tested, and reported (**Chapter 2**). This interpretation is supported by findings from a community-based RSV mortality study conducted in an urban slum in Buenos Aires, Argentina, which described two distinct settings of RSV-related infant mortality: deaths occurring in medical facilities, often in the context of severe complications, and deaths occurring in the community, which were closely associated with social inequalities and characterized by mild or mixed clinical presentations and underestimation of disease severity by both parents and physicians, likely reflecting the paucity of overt clinical signs [28].

Taken together with the sensitivity analyses presented in **Chapter 2**, the observed differences between in-hospital and community RSV-related deaths likely reflect limitations of hospital-based data in capturing the full spectrum of RSV disease, as well as survival bias. In healthcare settings, RSV-related deaths are primarily identified among infants with clear or severe clinical manifestations who survive long enough to be tested and diagnosed, whereas fatal infections with mild, atypical, or rapidly progressive presentations may go unrecognized and are therefore underrepresented. As a result, hospital-based data reflect only a subset of RSV-related mortality and may overestimate age at death compared with the true population-level burden.

This distinction has important implications for estimates of vaccine impact. As noted earlier, the global RSV burden estimates by Shi et al. (2017)[12] and their update by Li et al. (2022)[5] are widely used as core inputs for RSV impact modelling and cost-effectiveness analyses, including those informing Gavi decision-making. The prospective data model suggest that in Gavi-eligible settings, 77% of deaths occur in the first 6 months of life. **Applying the Li et al. estimates, which assign only 45% of deaths to this age group, would markedly underestimate the mortality burden among infants younger than six months.** Under the assumption that vaccine-induced protection is limited to the first 6 months of life, this would imply that the potential vaccine-preventable mortality burden, and hence estimated vaccine impact, could be approximately 1.7-fold higher than what would be expected based on the Li et al. estimates. Therefore, accurate burden estimates are essential to ensure

that global immunization and prioritization decisions are based on a complete and reliable assessment of RSV mortality [11].

In summary, studies that rely on retrospective mortality data tend to overestimate the age at death, leading to an underestimation of both potential vaccine impact and cost-effectiveness.

## Part II Potential impact of maternal vaccination

*Maternal antibody transfer model*

Building on Scheltema et al. [22], in **Chapter 4,** we updated, optimized and extended the original maternal vaccination equation-based model of maternal antibody transfer. Optimal vaccination windows were identified at around 24–27 weeks' gestation depending on subtype and country setting. By incorporating country-specific probability distributions for the timing of maternal vaccination, we estimated a theoretical maximum impact of 80–87% for RSV-A (and 71–76% for RSV-B) on RSV-related mortality occurring within the first 6 months of life. This modeling work was performed before the work of **Chapter 3**. Incorporating the revised, younger age distribution would likely increase the estimated proportion of cases averted by maternal vaccination, resulting in a larger projected number of prevented cases.

Our model explicitly incorporates antenatal care (ANC) timing and coverage by using the country-specific distribution of ANC visits within the 24–36-week gestational window as a proxy for the timing of maternal vaccination. **After incorporating realistic vaccine-coverage per country, the potential impact ranged from 83% for Maldives to 33% for Burundi and a weighted average of 55-63%.** We did not account for other important programmatic constraints, including cost, health-system capacity, vaccine delivery feasibility beyond ANC attendance, alignment with RSV seasonality, and vaccine pricing.

Furthermore, these projections remain sensitive to key immunological assumptions embedded in the model. Potentially the most influential assumption in our model is the choice of the protection threshold. Because this threshold directly determines the duration of protection, even small changes in its value can substantially alter the estimated number of protected days and, consequently, the projected vaccine impact. We assumed that palivizumab serum titers, or corresponding RSV neutralizing antibody titers equal to or above 100 µg/ml, could avert (or at least postpone) RSV-related mortality. While this represents the best-established quantitative association with protection to date, there is currently no validated correlate of protection for RSV vaccines [29]. **Establishing a robust and generalizable correlate of protection is therefore essential to reduce uncertainty in predictions of the impact of maternal RSV vaccination.**

*Consistence with emerging real-world evidence*

**Recent post-implementation data reveal a major impact in reducing the burden of RSV disease in infants following introduction of the maternal vaccine** [30]. Argentina was the first country to introduce the RSV prefusion F protein (RSVpreF) vaccine as the primary intervention in a national RSV immunization program in March 2024, and multiple studies have been executed to estimate its real-world effectiveness [31–33]. These studies show that the RSVpreF vaccination was safe and highly effective. No deaths were recorded among these infants, however the reported vaccine effectiveness for Pediatric Intensive Care Unit admission was 87.2% (95%CI, 52.6–97.0) in infants under 6 months [31]. **This aligns closely with our Chapter 3 predictions for RSV-A (80–87%) under the assumption of full vaccination**.

*Implications of vaccination timing restrictions*

There have been concerns about the safety of the RSVpreF vaccine in the RSV-field, as the phase 3 trial of the maternal RSVpreF vaccine showed a numerical (albeit statistically non-significant) imbalance in preterm births between study groups, specifically in two UMICs during the COVID-19 Delta and Omicron waves [34]. Regulatory agencies have interpreted this signal differently, with the EMA ignoring this statistically non-significant signal and recommending vaccination at 24–36 weeks gestational age, in line with trial protocols, while the FDA restricted its usage to 32–36 weeks gestational age because of concerns about increased risk of preterm birth.

In **Chapter 5**, we examined the consequence of the regulatory divergence between the FDA and EMA regarding the recommended timing of RSV maternal vaccination. Drawing on the modelling work in **Chapter 4**, we concluded that if early vaccination does not increase the risk of preterm birth, the optimal window for administration would be between 24 and 27 weeks of gestation. **Given that approximately two weeks are required to mount an effective immune response, the FDA restriction to vaccinate only after 32 weeks would imply that infants born before 34 weeks would receive little to no protection. We estimated that this restriction could reduce the global mortality impact of the vaccine by approximately 12%, corresponding to about 3,300 additional deaths annually.**

In practice, the overall impact of the strict FDA regulations is likely to be even larger. A narrower vaccination window also reduces the probability of reaching pregnant individuals during routine antenatal care, as there are fewer opportunities to “catch” individuals within the eligible gestational period, particularly in settings with

infrequent or late antenatal visits. As a result, overall vaccine coverage would likely be lower than assumed in our analysis, leading to lower vaccine impact.

*Post-regulatory evidence and recommendations for LMICs*

In line with our estimates, a recent modelling study found that the direct benefits of the RSV maternal vaccine in South Africa outweighed the risks when vaccination was provided from 28 weeks onwards in 98% of simulations [35]. However, LMICs have been under-represented in RSV vaccine research, resulting in limited data on safety and effectiveness for these settings [30]. As a precaution, SAGE has recommended administering the vaccine in the third trimester, particularly in LMIC contexts. Looking ahead, both the Global Advisory Committee on Vaccine Safety and SAGE have emphasized the need for continued post-implementation pharmacovigilance. In addition, a forthcoming phase 4 trial (NCT06955728)[36] across four African countries will further assess vaccine efficacy and safety.

In summary, we estimated that the RSVpreF maternal vaccine could reduce RSV-related mortality in LMICs in the first 6 months of life by 55-63%, with its overall impact strongly driven by ANC coverage. Delaying administration until the third trimester would restrict the vaccine's coverage and therefore effectiveness.

## Part III: Antibody specific kinetics

*Antibody half-life in preterm versus full-term infants*

In **Chapter 6** we investigated the antibody half-life of preterm *versus* full-term infants. At first glance, we found a prolonged half-life of RSV-neutralizing antibodies in preterm infants. When diving deeper into the data, we found that this effect was almost completely driven by an inverse association between cord blood antibody levels and antibody half-life, i.e. postnatal antibody half-life depends on its initial concentration (i.e. cord blood level), with higher titers declining more rapidly. If this association would also hold for vaccine-induced antibodies, we would predict that the difference in vaccine protection between preterm and term infants would be smaller than estimated in **Chapter 4**.

Quantifying the precise vaccine impact in preterm and term infants was outside the scope of this modelling extension, because extrapolation of our findings to vaccine scenarios with high antibody levels not observed in our cohort would result in imprecise estimates. Despite this limitation, the analysis provides an important conceptual insight. Increasing maternal antibody levels, and consequently cord blood antibody levels, is likely to reduce the difference in protection between preterm and term infants. For illustration, a two-fold increase in maternal antibody levels resulted in preterm infants having 73 fewer days of protection than term infants, whereas a ten-fold increase reduced this difference to 28 days.

*Parallels between antibody transfer and decay mechanisms*

Interestingly, similar to the pattern observed in our antibody data, a review of the literature shows that placental IgG transport is dependent on IgG concentration [37,38]. Although neonatal IgG levels generally correlate with maternal IgG levels, several studies found more efficient transplacental transfer (i.e. higher ratio of cord:maternal antibody concentration) when maternal IgG concentration, both total and antigen-specific, was lower. [39] [38]Both IgG transport over the placenta and IgG recycling in the bloodstream are governed by the FcRn receptor [39] (**Box 1**). A possible explanation for the concentration-dependent half-life and maternal transfer efficiency of IgG antibodies is that the pool of FcRn is limited, and that antibody transfer and recycling are therefore saturable processes [40–44]. When FcRn is fully saturated, the unbound IgG is cleared, primarily through lysosomal degradation, resulting in a relatively high proportion of IgG being degraded (i.e. high fractional catabolic rate) (**Figure 3**). This is referred to as the concentration–catabolism effect. This hypothesis has been strengthened by animal studies showing that higher than normal expression levels of FcRn reduced exogenous IgG catabolism in transgenic animals, resulting in prolonged IgG half-life [45].

**Box 1: FcRn: the neonatal Fc receptor**

During pregnancy, IgG is the only type of antibody that is actively passed from a mother to her unborn baby, providing the baby with short-term protection after birth [39]. This transfer is made possible by a specialized transport protein called the neonatal Fc receptor (FcRn). FcRn is located in the placenta (specifically in the syncytiotrophoblasts, which form a barrier between the fetus and the mother) and acts like a carrier, transferring IgG antibodies from the mother's bloodstream through the placenta to the fetus (**Figure 2A**). As pregnancy progresses, this transfer becomes more efficient, allowing increasing amounts of protective antibodies to reach the fetus. These maternal antibodies help protect newborns during the early months of life, before their own immune system is fully developed.

FcRn also protects IgG throughout life by preventing these antibodies from being broken down too quickly [39]. Certain cells take up IgG from the blood, and FcRn acts like a recycling system: it binds to IgG inside the cell and returns it into the circulation, thereby preventing lysosomal degradation (**Figure 2B**). Proteins that do not use this recycling system are broken down more rapidly. Because of this role, FcRn has become an important target for new medical treatments. For example, nirsevimab is designed to stay in the body for a long time by making better use of this recycling process [59]. A small change in its structure allows it to bind more strongly to FcRn inside cells, so less of it is broken down and more is returned to the bloodstream. As a result, nirsevimab remains effective in the body for longer than typical antibodies.

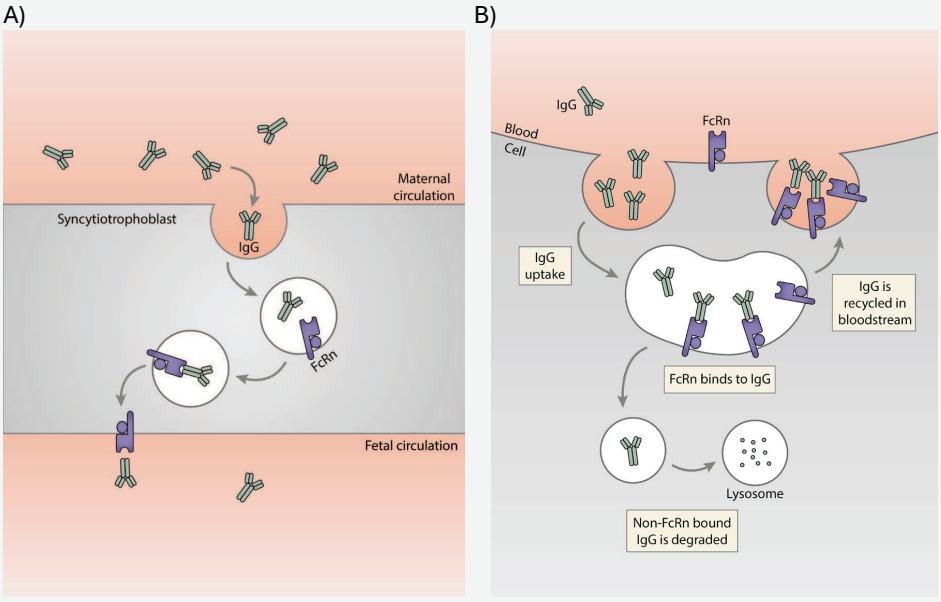



**Figure 2**: FcRn mediates the perinatal transfer of IgG (A) and IgG recycling in the bloodstream (B). Figures adapted from [39].

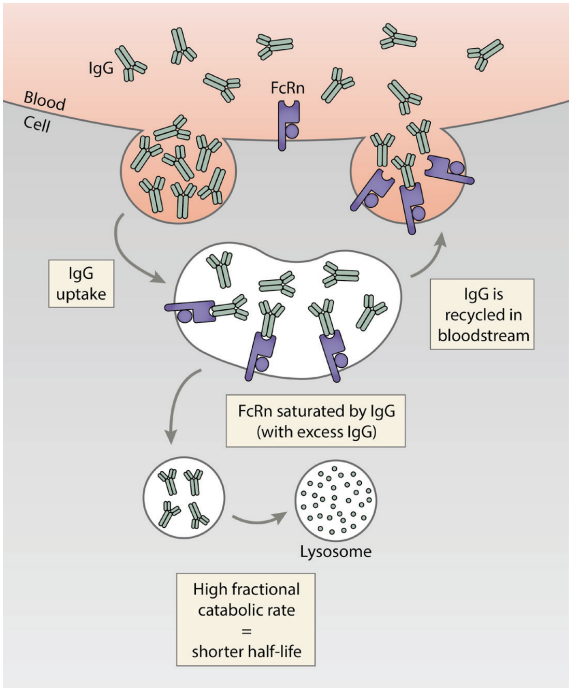



**Figure 3**: High fractional catabolic rate resulting from FcRn saturation during IgG recycling. Figure adapted from [45]

*Population-level differences in transplacental IgG transfer*

These mechanisms are especially relevant in settings where maternal IgG levels are high. In many African populations, greater infectious exposure leads to elevated IgG concentrations, and multiple studies have reported transplacental transfer in these contexts [37,38,46]. In line with this, the Novavax trial [23] showed slightly reduced transplacental transfer efficiency of RSV-specific antibodies in infants from LMICs compared to high-income countries, alongside longer apparent antibody half-lives. Differences in IgG glycosylation, which influences selective antibody transfer, have also been reported across ethnic groups, geographic settings, and health states [47]. Malaria and HIV, which are particularly prevalent in developing countries, have been associated with reduced transplacental transfer. Clinical trials of vaccination in pregnancy typically enroll healthy women, without chronic infections or co-morbidities, constraining the relevance of trial findings to real-world settings[38]. For these reasons, vaccine impact models that rely on transfer ratios derived from clinical trials conducted in Western cohorts are likely to overestimate antibody transfer in LMICs.

*Implications for maternal vaccination*

The findings of this thesis challenge the assumption that antibody half-life is fixed over time and consistent across individuals. Likewise, antibody transfer may depend on factors beyond gestational age alone. In line with this view, Wessel and Dolatshahi presented a mechanistic model describing the biological pathways by which maternal antibodies reach the fetus [47,48]. Three factors were identified as key determinants of transfer: the mother's antibody pool (both quantity and quality); the level of Fc receptor expression in the placenta; and the binding strength between antibodies and these receptors. When receptor availability becomes limited, particularly later in pregnancy, antibodies effectively compete for transport. **Future research should extend this mechanistic framework to antibody half-life dynamics, explicitly accounting for the quantity and quality of the neonatal antibody pool at birth, neonatal FcRn expression levels, and antibody–Fc receptor binding strength.**

*Placental abnormalities and antibody transfer*

In **Chapter 7,** we explored the potential relationship between placental abnormalities and transplacental antibody transfer. We found no evidence supporting an association. However, this finding should be interpreted with caution. The study was exploratory and limited by a small sample size of 85 participants, resulting in low statistical power, particularly given that only mild placental abnormalities were observed. In addition, cord:maternal antibody concentration ratios were used as the measure of transplacental antibody transfer. As discussed above, the transfer ratio is inherently influenced by maternal antibody concentrations, introducing additional variability that may have further reduced our ability to detect a true association.

Summarizing, improving understanding and prediction of vaccine effectiveness requires insights into the mechanisms governing antibody half-life and transplacental transfer. Key factors likely include antibody quantity and quality, FcRn expression, and FcRn binding strength, as well as how these vary across populations and (gestational) age. Such insights would advance vaccinology and, when incorporated into vaccine effectiveness models, increase expected protection in preterm infants and consequently narrow the gap with full-term infants.

## Part IV: Clinical trial design

*Efficacy shortfalls in LMICs*

Clinical trials may show levels of efficacy that do not fully translate into real-life effectiveness, especially in LMICs [49,50]. As seen with the rotavirus vaccine (98% efficacy in Finland and the USA, contrasted with 64% and 51% in sub-Saharan Africa and Asia, regions that were not included in the pivotal trials) [51] and several other vaccines and therapeutics, efficacy estimates from high-income country trials frequently overstate real-world effectiveness in LMICs [50,51]. **When pivotal trials exclude high-burden settings, post-licensure impact estimates rely heavily on extrapolation, which can introduce substantial uncertainty and reduce the reliability of policy decisions.**

*Designing randomized controlled trials (RCTs) to better inform post-licensure decision-making*

**Chapter 8** focuses on designing RCTs to better inform post-licensure decision-making. Because trials are rarely powered to detect subgroup- or country-specific effects, meta-regression models may provide a framework for comparing results across sites and producing more robust, context-specific estimates of efficacy. However, this approach requires sufficient data from diverse settings and broadly available predictors of heterogeneity in vaccine performance. **Discussions highlighted a persistent misalignment between the evidence needed for licensure and the evidence required post-licensure to guide vaccine deployment, particularly in LMICs.** When selecting study sites, researchers must balance the desire for geographic diversity with practical constraints related to logistics, cost, and participant recruitment.

*Ethical considerations*

Beyond methodological needs, there are strong ethical arguments for expanding trial participation in countries with high disease burden [50]. **The principles of social value and fair inclusion imply that populations most affected by the diseases targeted by new vaccines and medicines should have meaningful opportunities to contribute to (and benefit from) the evidence base.** Current geographic imbalances in trial conduct risk undermining equity by producing data that are less applicable to disadvantaged populations. Aligning site selection with global disease burden and improving transparency in reporting are therefore both ethically required and scientifically advantageous.

*Optimizing multiple endpoint selection in clinical trials*

To meet regulatory requirements, interventions must demonstrate efficacy against a prespecified primary outcome in randomized clinical trials. However, when multiple clinically relevant outcomes exist, selecting a single primary endpoint can be challenging, and incorporating information across outcomes may improve statistical power. In **Chapter 9**, we introduced a novel permutation-based test, a weighted average of individual endpoint test statistics (wavP), to evaluate intervention efficacy using multiple endpoints. **Our findings showed that this weighted permutation approach can increase power while maintaining appropriate type I error control under conditions reflective of real-world RSV clinical trials. Key questions remain about how results based on multiple endpoints would inform licensure and labeling**, given that manufacturers and regulators typically need a clear, single indication of use.

In summary, modelling can play a critical role in optimizing clinical trial design by informing endpoint selection (e.g., mortality, severe disease, hospitalization) and site selection, producing evidence that is better suited for integration into vaccine impact models.

## Recommendations for post-licensure impact modelling of maternal RSV vaccination

In July 2025, Gavi, The Vaccine Alliance announced its decision to support maternal RSV vaccination programs in eligible LMICs, marking a major advance in global RSV prevention [52]. While Gavi's current investment case focuses on maternal vaccination due to the high cost of licensed monoclonal antibodies, more affordable long-acting monoclonal antibodies are in development [13,53]. As maternal vaccination and monoclonal antibodies differ fundamentally in delivery platforms and populations reached, and head-to-head trials are unlikely, post-licensure mathematical modelling is essential to compare their potential impact under realistic health-system and program assumptions, particularly in LMIC settings. Drawing on the findings of this thesis and anticipated evidence gaps, the following recommendations outline key priorities for strengthening post-licensure impact modelling of maternal RSV vaccination.

1. **Update age-at-death distributions using population-representative data**
   The static impact model currently used by Gavi, The Vaccine Alliance [53] relies on age-specific RSV burden and mortality estimates from Li et al. (2022). Incorporating more accurate age-at-death distributions, such as those presented in **Chapter 3**, would substantially improve model validity, particularly for interventions targeting the neonatal period.

2. **Use immunologically informed models to translate efficacy into effectiveness**
   Immunologically informed models, such as the framework developed in **Chapter 4**, provide a principled approach to translating clinical efficacy into real-world effectiveness. By explicitly modelling gestational age at vaccination, maternal antibody levels, placental transfer, and infant antibody decay, these models can generate population-specific protection estimates and identify groups at risk of reduced effectiveness.

3. **Use antibody kinetics from vaccinated populations to refine immunologically informed models**
   Updated data on maternal–infant antibody transfer and infant antibody decay following maternal RSV vaccination are needed, especially from LMIC settings, given evidence that both processes are concentration dependent. Quantifying these dynamics across settings would refine assumptions on the duration and

magnitude of protection and reduce uncertainty in effectiveness estimates for key risk groups, including preterm infants.

**4. Strengthen evidence on correlates of protection**

Uncertainty around correlates of protection remains a major limitation for RSV impact modelling. Establishing and validating robust correlates is critical to improve estimates of the magnitude and duration of vaccine-induced protection and to narrow uncertainty in impact projections.

**5. Explicit representation of programmatic feasibility**

Maternal vaccination models should incorporate realistic ANC attendance patterns, gestational age–specific eligibility, and vaccination timing relative to pregnancy and RSV seasonality. Although outside the scope of this thesis, in many LMICs, additional implementation challenges, such as difficulties maintaining vaccines at required temperatures during storage and transport (cold-chain requirements), particularly in remote areas, unpredictable seasonal patterns, irregular antenatal care timing, and high vaccine hesitancy may further constrain delivery [30]. Given that maternal RSV vaccination and long-acting monoclonal antibodies rely on fundamentally different delivery platforms [13,53], comparative modelling should focus on feasible, programmatic impact under real-world conditions rather than on theoretical maximum efficacy.

Integrating maternal RSV vaccination into ANC services also presents an opportunity to strengthen this platform more broadly, with potential spillover benefits for maternal, newborn, and child health [13]. Several studies [54–57] indicate a strong correlation between maternal health interventions (ANC visits, BCG and tetanus vaccination during pregnancy), and full immunization in children. Future modelling should quantify the indirect benefits of ANC strengthening to enable comprehensive comparison of maternal vaccination and monoclonal antibody strategies in terms of childhood mortality impact.

**6. Combining immunologically informed and transmission-based models**

Where this thesis focuses primarily on RSV-associated mortality, future work should combine immunologically informed models with transmission-based models that explicitly represent RSV transmission dynamics and heterogeneity in disease risk and severity [22], such as the compartmental framework recently published by Zheng et al. [58]. Such integrated approaches would provide more comprehensive estimates of vaccine impact at the population level.

7. **Design clinical trials to support post-licensure impact modeling**
   Maternal RSV vaccine trials should include a diversity of high-burden LMIC settings to reduce reliance on extrapolation in post-licensure impact models and to better inform post-licensure decision-making. The forthcoming Phase 4 trial [36] enrolling participants across four African countries will be critical for updating efficacy estimates in LMIC settings.

## CLOSING REMARKS

In this thesis, I have shown that reliance on retrospective and hospital-based mortality data leads to systematic overestimation of the age at death and, consequently, underestimation of the potential impact of maternal RSV vaccination. I further demonstrated that translating vaccine efficacy observed in clinical trials into real-world effectiveness requires a mechanistic understanding of antibody half-life and transplacental transfer. In particular, antibody quantity emerged as a key determinant shaping antibody kinetics, introducing non-linear dynamics that add complexity to vaccine impact modelling. Finally, this thesis highlights the role of modelling in informing clinical trial design, especially endpoint and site selection, to generate evidence that is better aligned with the needs of post-licensure impact estimation and policy decision-making.

As new data from early-adopting countries and Phase 4 studies become available, impact models should be updated iteratively. Modelling frameworks that combine improved burden estimates, immunologically informed protection, and realistic delivery assumptions will be critical to guide the optimal deployment of maternal RSV vaccination alongside alternative and complementary prevention strategies as the RSV prevention landscape continues to evolve. To support evidence-based prioritization by policymakers such as Gavi, effectiveness estimates must feed directly into cost-effectiveness analyses, integrating realistic effectiveness with programmatic costs to identify strategies that maximize health impact and value for money.

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# Appendices



## Aim of this thesis

In the past three years, new respiratory syncytial virus (RSV) vaccines and monoclonal antibodies have become available in high-income countries. However, without financial support from Gavi, the Vaccine Alliance, these products remain out of reach for most low- and middle-income countries (LMICs). The main objective of this thesis was to inform cost-effectiveness modellers and decision-makers about the potential impact of maternal vaccination on RSV-related mortality in LMICs. To achieve this, we refined an existing mathematical model and updated and validated its key inputs and underlying assumptions.

## Outline of this thesis

**Part I** characterizes age-at-death distributions across infancy, which are critical for estimating the proportion of mortality that can be averted.

In **Chapter 2,** we aimed to describe global community pediatric deaths under 6 months and to compare this group with in-hospital deaths in upper-middle-income countries (UMICs) and LMICs. We observed that children dying in the community were significantly younger than those dying in hospital. However, in a sensitivity analysis restricted to data from community-based mortality studies, the proportion of neonates was similar among the in-hospital and community deaths. This suggested that the observed difference in the main analysis was largely driven by differences in data collection methodology.

In **Chapter 3**, we further examined how differences in data collection methods within the RSV GOLD mortality database (RSV Global Online Mortality Database) influence estimates of age at death, focusing specifically on data from Gavi-eligible countries. Data collection methodology emerged as a significant predictor of age at death; the prospective community mortality studies, largely based on systematic postmortem sampling, yielded substantially lower age at death estimates than other registry data. We concluded that studies that rely on retrospective mortality data tend to overestimate the age at death, leading to an underestimation of both potential vaccine impact and cost-effectiveness.

**Part II** integrates the available epidemiological and immunological evidence and clinical trial inputs at the time into an impact modelling framework, to estimate the potential impact of maternal vaccination.

In **Chapter 4,** we updated, optimized and extended the original maternal vaccination equation-based model of maternal antibody transfer. Optimal vaccination windows were identified at around 24–27 weeks' gestation depending on subtype and country setting. Our model explicitly incorporates antenatal care (ANC) timing and coverage by using the country-specific distribution of ANC visits within the 24–36-week

gestational window as a proxy for the timing of maternal vaccination. After incorporating realistic, country-specific vaccine coverage, the potential impact varied by country, ranging from 33% to 83%, with a weighted average of 55–63%.

Drawing on the modelling work in the previous chapter, in **Chapter 5**, we examined the consequence of the regulatory divergence between the Food and Drug Administration (FDA, in the USA) and the European Medicines Agency (EMA) regarding the recommended timing of RSV maternal vaccination. We estimated that adoption of the more restricted window for administration in LMICs could reduce the global mortality impact of the vaccine by approximately 12%, corresponding to about 3,300 additional deaths annually.

**Part III** tests and refines the immunological assumptions in the maternal vaccination model by quantifying antibody decay in early life, and studying the relation between placental health and maternal antibody transfer.

In **Chapter 6,** we investigated the RSV-specific antibody half-life in preterm *versus* full-term infants. At first glance, we found a prolonged half-life of RSV-neutralizing antibodies in preterm infants. When diving deeper into the data, we found that this effect was almost completely driven by an inverse association between cord blood antibody levels and antibody half-life, i.e. postnatal antibody half-life depended on antibody concentration at birth, with higher antibody titers declining more rapidly. If this association also holds for vaccine-induced antibodies, we predict that the difference in vaccine protection between preterm and term infants would be smaller than currently anticipated.

In **Chapter 7,** we explored the potential relationship between placental abnormalities and transplacental antibody transfer. We found no evidence supporting an association. However, this finding should be interpreted with caution as the study was exploratory and limited to a small sample size, resulting in low statistical power, particularly given that only mild placental abnormalities were observed.

**Part IV** reflects on how optimizing clinical trial design can generate outputs that are more readily usable in impact models, reducing reliance on extrapolation across settings and strengthening the link between trial evidence and impact estimation.

**Chapter 8** summarizes a meeting held during a scientific congress that focused on critical aspects of clinical trial design for RSV preventative therapies. Discussions highlighted a persistent misalignment between the evidence needed for licensure and the evidence required post-licensure to guide vaccine deployment, particularly in LMICs. A key debate was whether to prioritize efficiency for faster, lower-cost licensure or larger, more robust trials to inform country- or region-specific decisions.

In **Chapter 9**, we introduced a novel permutation-based test, a weighted average of individual endpoint test statistics, to evaluate intervention efficacy using multiple endpoints. Our findings show that this weighted permutation approach can increase power while maintaining appropriate type I error control under conditions reflective of real-world RSV clinical trials. Key questions remain about how results based on multiple endpoints would inform licensure and labelling, given that manufacturers and regulators typically need a clear, single indication of use.

In the **General Discussion,** we reviewed lessons learned from the work in this thesis. We propose recommendations for post-licensure impact modelling of maternal RSV vaccination. We concluded that modelling frameworks that combine improved burden estimates, immunologically informed protection, and realistic delivery assumptions will be critical to guide the optimal deployment of maternal RSV vaccination alongside alternative and complementary prevention strategies as the RSV prevention landscape continues to evolve.

## Doelstellingen van dit proefschrift

In de afgelopen drie jaar zijn nieuwe RSV-vaccins en monoklonale antistoffen beschikbaar gekomen in hoge-inkomenslanden. Voor de meeste lage- en middeninkomenslanden (LMICs) blijven deze middelen echter buiten bereik zonder financiële ondersteuning van *Gavi, the Vaccine Alliance*. Dit proefschrift had als doel om kosteneffectiviteitsmodelleurs en beleidsmakers beter inzicht te geven in de mogelijke impact van maternale vaccinatie op RSV-gerelateerde sterfte in deze landen. Hiervoor hebben we een bestaand wiskundig model verfijnd en de belangrijkste aannames en invoergegevens geactualiseerd en gevalideerd.

## Indeling van dit proefschrift

**Deel I** richt zich op de leeftijd waarop kinderen overlijden aan RSV in de eerste levensmaanden, omdat dit een belangrijke factor is bij het inschatten hoeveel sterfte door vaccinatie voorkomen kan worden.

In **Hoofdstuk 2** beschrijven we sterfte onder kinderen jonger dan 6 maanden in LMICs die buiten het ziekenhuis plaatsvindt, en vergelijken we deze met sterfte in het ziekenhuis. Hieruit blijkt dat kinderen die *buiten* het ziekenhuis overlijden gemiddeld jonger zijn dan kinderen die *in* het ziekenhuis overlijden. Wanneer we de analyse beperken tot studies die specifiek zijn opgezet om sterfte in de gemeenschap in kaart te brengen, blijkt het aandeel pasgeborenen (jonger dan 1 maand) echter vergelijkbaar tussen beide groepen. Dit wijst erop dat het gevonden verschil grotendeels wordt veroorzaakt door verschillen in de manier waarop de data zijn verzameld.

In **Hoofdstuk 3** verdiepen we dit door te onderzoeken hoe verschillende dataverzamelingsmethoden binnen de *RSV GOLD-database* (*RSV Global Online Mortality Database*) de geschatte leeftijd bij overlijden beïnvloeden, waarbij we ons hebben beperkt tot landen die in aanmerking komen voor *Gavi*-ondersteuning. De methode van dataverzameling blijkt inderdaad een belangrijke rol te spelen. Prospectieve studies die specifiek zijn opgezet om sterfte in de gemeenschap in kaart te brengen, en vaak gebruikmaken van systematisch *post-mortem*-onderzoek, laten significant lagere leeftijden bij overlijden zien dan andere databronnen. Studies die gebruikmaken van retrospectieve gegevens lijken de gemiddelde leeftijd bij overlijden te overschatten, wat kan leiden tot een onderschatting van zowel de potentiële impact als de kosteneffectiviteit van vaccins.

**Deel II** combineert epidemiologische en immunologische kennis met gegevens uit klinische studies in een wiskundig model om de mogelijke impact van vaccinatie tijdens de zwangerschap op kindersterfte te schatten.

In **Hoofdstuk 4** hebben we het bestaande model voor zwangerschapsvaccinatie verder ontwikkeld en uitgebreid. We vonden dat het optimale moment voor RSV-

vaccinatie rond 24 tot 27 weken zwangerschap ligt, afhankelijk van het land en het virussubtype. Het model houdt rekening met verschillen tussen landen in het gebruik van prenatale zorg, waarbij we aannemen dat zwangere vrouwen het vaccin ontvangen tijdens contactmomenten met de prenatale zorg. Wanneer rekening wordt gehouden met een realistische vaccinatiegraad per land, varieert de geschatte afname in sterfte van 33% tot 83%, met een gemiddelde van 55–63%.

Voortbouwend op het werk uit het voorgaande hoofdstuk onderzoeken we in **Hoofdstuk 5** de gevolgen van verschillen in aanbevelingen tussen de *Food and Drug Administration* (FDA) en de *European Medicines Agency* (EMA) met betrekking tot het toegestane vaccinatieschema. Als vaccinatie in LMICs wordt beperkt tot een later moment in de zwangerschap, kan dit de wereldwijde impact op sterfte met ongeveer 12% verminderen, wat neerkomt op circa 3.300 extra sterfgevallen per jaar.

**Deel III** test en verfijnt de immunologische aannames in het maternale vaccinatiemodel, zoals hoe snel antistofniveaus afnemen bij jonge kinderen en hoe de gezondheid van de placenta de overdracht van maternale antistoffen beïnvloedt.

In **Hoofdstuk 6** onderzoeken we de halfwaardetijd van antistoffen bij prematuur en *à term* geboren kinderen. Op het eerste gezicht lijken premature hun (aanvankelijk lagere hoeveelheid) antistoffen langer te behouden. Nadere analyse laat echter zien dat dit vooral komt doordat kinderen met hogere antistofniveaus bij geboorte deze sneller verliezen. Als dit ook geldt voor vaccin-geïnduceerde antistoffen, is het verschil in bescherming tussen premature en *à term* geboren kinderen waarschijnlijk kleiner dan gedacht.

In **Hoofdstuk 7** onderzoeken we of afwijkingen aan de placenta invloed hebben op de overdracht van antistoffen van moeder op kind. We vinden geen duidelijk verband, maar deze resultaten moeten voorzichtig worden geïnterpreteerd vanwege de kleine onderzoeksgroep en beperkte statistische zekerheid.

**Deel IV** reflecteert op hoe klinisch onderzoek beter kan worden ontworpen zodat de resultaten beter bruikbaar zijn voor impactmodellen en beleidsbeslissingen.

**Hoofdstuk 8** beschrijft een besloten bijeenkomst die tijdens een wetenschappelijk congres plaatsvond en gericht was op de opzet van klinische studies naar RSV-preventie. Hieruit komt naar voren dat er vaak een kloof bestaat tussen het bewijs dat nodig is voor de toelating van vaccins en het bewijs dat nodig is om ze effectief in te kunnen zetten, vooral in LMIC's. Daarbij speelt de vraag of studies vooral snel en efficiënt moeten zijn, of juist groter en uitgebreider om beleidsbeslissingen beter te ondersteunen.

In **Hoofdstuk 9** introduceren we een nieuwe statistische methode om de effectiviteit van interventies met meerdere uitkomstmaten te analyseren. Deze methode kan de

kans vergroten om effecten aan te tonen, zonder het risico op fout-positieve conclusies te verhogen. Tegelijkertijd blijft onduidelijk hoe resultaten op basis van meerdere uitkomstmaten kunnen worden gebruikt bij registratie en indicatiestelling, waarbij vaak één duidelijke uitkomstmaat wordt vereist.

In de **Algemene Discussie** bundelen we de belangrijkste inzichten uit dit proefschrift en doen we aanbevelingen voor toekomstige impactmodellen van maternale RSV-vaccinatie. We concluderen dat modellen die verbeterde schattingen van de ziektelast combineren met immunologisch geïnformeerde bescherming en realistische aannames over de implementatie, essentieel zijn om de inzet van RSV-vaccinatie en andere preventiestrategieën optimaal vorm te geven.

## Publications in this thesis

### *Published*

Mazur NI, Löwensteyn YN, **Willemsen JE**, Gill CJ, Forman L, Mwananyanda LM, Blau DM, Breiman RF, Madhi SA, Mahtab S, Gurley ES. Global respiratory syncytial virus–related infant community deaths. ***Clinical Infectious Diseases***. 2021 Sep 1;73(Supplement_3):S229-37.

**Willemsen JE**, Vernooij FS, Shaaban FL, Chikoti C, Bont LJ, Drylewicz J, RSV GOLD study group. Characteristics of inpatient and outpatient respiratory syncytial virus mortality in Gavi-eligible countries. ***Vaccine: X***. 2024 Oct 1;20:100554.

**Willemsen JE**, Borghans JA, Bont LJ, Drylewicz J. Maternal vaccination against RSV can substantially reduce childhood mortality in low-income and middle-income countries: a mathematical modeling study. ***Vaccine: X***. 2023 Dec 1;15:100379.

**Willemsen JE**, Borghans JA, Bont LJ, Drylewicz J. Disagreement FDA and EMA on RSV maternal vaccination: possible consequence for global mortality. ***The Pediatric infectious disease journal***. 2024 Jan 1;43(1):e1-2.

Prunas O, **Willemsen J** (shared first authorship), Warren JL, Bont L, Schwartz JL, Atwell J, Begier E, Dean N, Hirsch I, Karron R, Klugman K. Workshop on the design and use of clinical trials with multiple endpoints, with a focus on prevention of RSV. ***Vaccine: X***. 2024 Aug 1;19:100509.

Prunas O, **Willemsen JE** (shared first authorship), Bont L, Pitzer VE, Warren JL, Weinberger DM. Incorporating data from multiple endpoints in the analysis of clinical trials: example from RSV vaccines. ***Epidemiology***. 2024 Jan 1;35(1):103-12.

### *Submitted*

**Willemsen JE**, Phijffer EWEM (shared first authorship), Wildenbeest JG, Anderson LJ, Ha B, Sankaranarayanan R, Groenendaal F, Bloemenkamp KWM, Barghans JAM, Drylewicz J, Bont L. Low Cord Blood Titers Drive Prolonged Maternal RSV Antibody Half-Lives in Preterm Infants. Submitted

Phijffer EWEM, **Willemsen JE** (shared first authorship), Wildenbeest JG, Anderson LJ, Bloemenkamp KWM, Groenendaal F, Nikkels PGJ, Bont LJ. Placental histopathology and transplacental transfer of RSV specific neutralizing antibodies – the SILVER study. Submitted.

## Publications not in this thesis

Van De Schoot R, De Bruin J, Schram R, Zahedi P, De Boer J, Weijdema F, Kramer B, Huijts M, Hoogerwerf M, Ferdinands G, Harkema A, **Willemsen J**, Ma Y, Fang Q,

Hindriks S, Tummers L, Oberski DL. An open source machine learning framework for efficient and transparent systematic reviews. ***Nature machine intelligence***. 2021 Feb;3(2):125-33.

Van de Schoot R, Depaoli S, King R, Kramer B, Märtens K, Tadesse MG, Vannucci M, Gelman A, Veen D, **Willemsen J**, Yau C. Bayesian statistics and modelling. ***Nature Reviews Methods Primers***. 2021 Jan 14;1(1):1.

Löwensteyn YN, Nair H, Nunes MC, van Roessel I, Vernooij FS, **Willemsen J**, Bont LJ, Mazur NI, Thorburn K, Nunes M, Chawana R. Estimated impact of maternal vaccination on global paediatric influenza-related in-hospital mortality: A retrospective case series. ***EClinicalMedicine***. 2021 Jul 1;37.

Löwensteyn YN, Mazur NI, Nair H, **Willemsen JE**, van Thiel G, Bont L. Describing global pediatric RSV disease at intensive care units in GAVI-eligible countries using molecular point-of-care diagnostics: the RSV GOLD-III study protocol. ***BMC infectious diseases***. 2021 Aug 23;21(1):857.

Langedijk AC, Harding ER, Konya B, Vrancken B, Lebbink RJ, Evers A, **Willemsen J**, Lemey P, Bont LJ. A systematic review on global RSV genetic data: Identification of knowledge gaps. ***Reviews in medical virology***. 2022 May;32(3):e2284.

Löwensteyn YN, **Willemsen JE**, Mazur NI, Scheltema NM, Van Haastregt NC, Ten Buuren AA, Van Roessel I, Scheepmaker D, Nair H, Van De Ven PM, Bont LJ. Nosocomial RSV-related in-hospital mortality in children< 5 years: a global case series. ***The Pediatric infectious disease journal***. 2023 Jan 1;42(1):1-7.

**RSV GOLD – ICU Network Study Group**. Respiratory syncytial virus infection among children younger than 2 years admitted to a paediatric intensive care unit with extended severe acute respiratory infection in ten Gavi-eligible countries: the RSV GOLD—ICU Network study. The Lancet Global Health. 2024 Oct 1;12(10):e1611-9.

Löwensteyn YN, Mazur NI, Nair H, **Willemsen JE**, van Thiel G, Bont L. Describing global pediatric RSV disease at intensive care units in GAVI-eligible countries using molecular point-of-care diagnostics: the RSV GOLD-III study protocol. ***BMC infectious diseases***. 2021 Aug 23;21(1):857.

## PHD PORTFOLIO

*Courses*

| | |
|---|---|
| 2023 | PhD talent program, UMC Utrecht (1 EC) |
| 2020-2025 | University Utrecht, Graduate School of Life Sciences: Psychological Flexibility (1 EC), Achieving your Goals and Performing more Succesfully in your PhD (1 EC), Psychological Flexibility (1 EC), Academic Writing in English (1,8 EC), Giving Effective Presentations (0.6 EC), Responsible conduct of science year 1-4 (0.6 EC) |

*Conferences*

| | |
|---|---|
| 2021 | 5th Workshop on Virus Dynamics. Online (poster) |
| 2022 | 12th International RSV Symposium (RSV2022). Belfast (poster) |
| 2022 | UMC Research Day. Utrecht (poster) |
| 2022 | UBC Annual Symposium 2022. Utrecht (poster) |
| 2023 | 7th conference RSVVW'23. Lisbon (poster) |
| 2024 | Vaccine Symposium Utrecht 2024. Utrecht (oral presentation) |
| 2025 | 13th International RSV Symposium (RSV2025). Iguazu (poster) |

*Seminars*

| | |
|---|---|
| 2021 | PALISI bronchiolitis subgroup webinar (oral) |

*Grants and funding*

| | |
|---|---|
| 2025 | Young Investigator Awards (RSV2025) |

*Teaching*

| | |
|---|---|
| 2020 | Computational Immunology course (teaching assistant) |
| 2022 | Human T-lymphocyte dynamics (Klinische Technologie) Delft (teaching assistant) |
| 2023 | Computational Immunology course (teaching assistant) |

*Supervision*

| | |
|---|---|
| 2022 | Femke Vernooij (Master student Medicine Utrecht University); Scientific intership. *Global paediatric respiratory syncytial virus-related mortality: characterising differences between income groups in the RSV GOLD study* |
| 2020-2023 | Supervision of eight medical students working as assistant researchers for the RSV GOLD Database |

*Peer review*

PIDJ, The Lancet Infectious Diseases (2), Nature Microbiology, Influenza and Other Respiratory Viruses, Clinical Infectious Diseases, Vaccine, Archives of Disease in Childhood

*Outreach*

| | |
|---|---|
| 2023 | Co-organized a workshop on the design and use of clinical trials with multiple endpoints at the 7th conference RSVVW'23 |

## About the author

Joukje Willemsen was born on December 23rd, in Utrecht, the Netherlands. She inherited her strong sense of justice and drive to change the world for the better from her dad's side, and her sense of responsibility and analytical mind from her mom's side.

She grew up with her younger brother, Ben. She spent her early childhood in Utrecht, attended middle school in Leuth, and completed high school in Wageningen. She was 12 when her first half-sister, Eelkje, was born on her dad's side, and not long after, her dad's family grew further with the arrival of her other half-siblings: Roosje, Jaap, Paul, and Koen. From her mom's side, she has two older stepsisters, Neeltje and Noortje.

In 2014, she moved back to Utrecht to study Biomedical Sciences. However, after realizing it was not a good fit, she switched to Psychology in 2015. She particularly enjoyed the mandatory statistics courses and filled all her elective courses with statistics- and mathematics-related subjects. For her bachelor's thesis, she taught herself to code in R to investigate Bayesian and frequentist decision qualities, work that earned her the prize for best bachelor's thesis in the Faculty of Social Sciences that year. She graduated cum laude.

In 2018, she began a master's in Methodology and Statistics for the Behavioral, Biomedical and Social Sciences. During this time, she completed an internship at the Julius Centre, focusing on the incorporation of historical controls in clinical trials. She also began working as a Statistical Illustrator, primarily for ASReview, an open-source project that was newly launched at the time.

In 2020, she began a PhD at the Department of Pediatric Immunology and Infectious Diseases at the Wilhelmina Children's Hospital (WKZ), under the supervision of Prof. Dr. Louis Bont, Prof. Dr. Jose Borghans, and Dr. Julia Drylewicz. Her doctoral research focused on developing a mathematical model to assess the potential impact of maternal RSV vaccination. As part of her PhD, she also led the RSV GOLD project, a global RSV mortality database, funded by the Gates Foundation. In 2021, she spent four months joining the Yale School of Public Health Modeling Unit, where she initiated and maintained international research collaborations and formed lasting friendships. She returned for additional visits in 2022 and 2023. She further developed a project proposal and study protocol for a new observational study, successfully securing funding from Pfizer, which she co-led together with her colleague Emily Phijffer.

As of December 2025 Joukje is working as a statistician at the Trial and Data Centre at the Prinses Maxima Centre in Utrecht.

Since 2022 Joukje has been living with her partner (and now husband) Nick in Woerden, together with their to cats Saar and Piep. They spend much of their free time renovating their house. In 2024 their son Alfie was born and they are expecting a daughter late summer 2026.

## Dankwoord

Hoewel dit proefschrift geen officiële stellingen kent, wil ik er op de valreep toch nog één introduceren: *"It takes a village to get a PhD"*. Er zijn veel factoren die bijdragen aan een succesvol promotieonderzoek, maar misschien wel de belangrijkste zijn de mensen om je heen. Een promovendus heeft niet alleen intellectuele ondersteuning nodig, ook sociale steun is onmisbaar. En een groep gezellige collega's om je heen maken het werk lichter en de dagen leuker. En gelukkig had ik zo'n *village*. In de pagina's die volgen wil ik de mensen bedanken die daar deel van uitmaakten.

*Promotieteam*

Allereerst heb ik het geluk gehad dat ik een enorm betrokken promotieteam had. Een promotieteam dat zich niet alleen druk maakte over mijn research output, maar ook om mijn persoonlijke ontwikkeling, werkplezier en carrièrepad.

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carrière te volgen en zelfs professor te worden. Dank ook voor het vertrouwen dat je mij vroeg om de illustraties bij je oratie te maken; dat was een hele eer.

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*Paranimfen*

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*Collega's*

*BONT groep*

**Annefleur**, **Anneke, Aranka**, **Arthur**, **Bianca**, **Brigitte**, **Coco**, **Emily**, **Ewout**, **Farina**, **Jonne**, **Klasina**, **Lobke**, **Marie**, **Natalie**, **Neele**, **Rosalie**, **Sarah**, **Susanne**, **Yvette**, **Zazie**, **Joanne, Eveline**, **Loes**, **Lies**, **Brigitte**, **Astrid**, **Hanneke**, hartelijk dank voor de gezelligheid. Wat zijn jullie een leuke groep en stuk voor stuk lieve en bijzondere mensen!

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**Astrid**, heel veel dank voor je betrokkenheid bij alle PhD's in de groep! Wat een luxe dat je zo met ons meedacht en dat we met de meest uiteenlopende vragen bij jou terechtkonden. En wat ongelooflijk lief dat er na de geboorte van Alfie ineens een zelfgemaakt geboortebord in de tuin stond.

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*CTI groep*

Dear CTI colleagues, especially, **Alejandra, Aroosha, Daphne, Enno, Hidde, Rianne, Sophie, Thom**, thank you for the entertaining coffee's and lunches.

**Rianne**, wij hebben lief en leed gedeeld op onze PhD kamer. Van persoonlijke onderwerpen tot B&B vol liefde, met jou in de kamer was het altijd gezellig. Dank dat je altijd zo'n fijn luisterend oor bent geweest en meeleefde, wat er dan ook in mijn leven speelde. En dank dat je zo gul je "snackla" met ons deelde, waardoor er in geval van nood altijd koffiecupjes, zoetigheden of zelfs noodles beschikbaar waren.

*YSPH Public Health Modeling Unit*

Dear YSPH Public Health Modeling group, it has been a great pleasure getting to know you and your work. I very much enjoyed the down-to-earth and welcoming atmosphere: students and professors having coffee and lunch together in the office, getting to know each other's cultures, and cracking jokes.

**Dan**, first of all, thank you for the invitation to visit your group and for helping me obtain a special visa to travel, making it possible to come despite the official (COVID-related) border restrictions. I greatly appreciate your patience in helping me learn new infectious disease modeling techniques, as well as your willingness to brainstorm with me on other projects. I also very much enjoyed your enthusiasm for sharing American culture. Thank you for inviting me to a real "tailgate party" for the Yale–Harvard football game, and for taking Louis, Corine, me, and your colleagues to a fantastic "sea shanty pub." My time in New Haven was an absolute highlight of my PhD.

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**Ottavia**, our friendship is one of the most unexpected and wonderful things that came out of this PhD. Spending four months in a foreign country could have been very lonely, but you took me along and introduced me to all of your friends, making it one of the most social and enjoyable times of my life. We were born in different countries and speak different languages, yet from the moment we met I felt that we truly understood each other. I think we share similar personal struggles and insecurities, and that we view and experience the world in much the same way. We also share strikingly similar experiences in early motherhood, and it has been such a comfort to share these with each other. You have been a wonderful source of support over the past years. Ti voglio tanto bene.

*Silver studie team*

**Elles**, **Hilletje**, **Emily**, **Joanne**, met jullie was de Silver studie een feestje! **Elles**, met jouw warme en empathische uitstraling en sterke betrokkenheid was jij de perfecte onderzoeksverpleegkundige voor deze precaire taak. En wat heb je dat fantastisch gedaan. Wat hebben we tijdens het koffiedrinken heerlijk over zwangerschap en baby's kunnen kletsen. Nog gefeliciteerd met je kleindochter; de rol als oma is je op het lijf geschreven.

*Studenten*

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*Current colleagues*

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