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Infecties met het respiratoir syncytieel virus aan weerszijden van het leeftijdsspectrum
(met een samenvatting in het Nederlands)

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CHAPTER 1

General introduction

Respiratory syncytial virus infection

Respiratory syncytial virus (RSV) was first isolated in chimpanzees in 1956 where it was initially named "Chimpanzee Coryza Agent" or CCA [3]. A laboratory worker who was in close contact with the infected chimpanzees also developed respiratory symptoms paving the way to the discovery of this virus in causing respiratory infections in human beings [3-5]. RSV typically starts with an upper respiratory tract infection with symptoms including rhinorrhea or nasal congestion and cough [6]. Progression to the lower respiratory tract may occur in 3-5 days leading to inflammation of the bronchioles commonly known as bronchiolitis. Bronchiolitis is associated with tachypnea, wheezing, low grade fever and dyspnea which in turn can lead to reduced oral intake [7, 8]. An uncommon but alarming symptom of bronchiolitis is apnea which occurs in approximately 5% of bronchiolitis cases although young children and those born prematurely are at increased risk [8]. Dehydration, reduced feeding and hypoxia or respiratory failure are the main reasons for hospital admission in children with RSV bronchiolitis [9]. Treatment for severe RSV is mainly supportive by use of supplemental oxygen, fluids, feeding and respiratory support which are used to support the child while bridging the gap to natural clearance of the infection. Unfortunately, well over 60 years after the discovery of RSV, treatment or prevention is still lacking. Vaccine development has been seriously delayed by the failure of the first formalin-inactivated RSV vaccine which resulted in enhanced RSV disease (ERD) in those that received it [10, 11]. To date, only passive immunization using palivizumab (a humanized monoclonal antibody) is available for prevention of RSV infection. Due to high costs palivizumab is currently only reserved for high-risk groups in high-income countries. Because of the current lack of proper treatment or preventive therapies, vaccine development for RSV has picked up again in the past decades and multiple vaccine candidates are currently being developed [12].

RSV infection in the pediatric population

Epidemiology of RSV infection in healthy term born infants can roughly be summarized in the 'ten-percent rule' pyramid [Figure 1]. Infection with RSV happens to everyone, most often already during the first three years of life [13-17]. Ten percent of those infected children develop lower respiratory tract infection (LRTI) [18-20], while another ten percent of those children with a LRTI require RSV hospitalization (RSVH) for secondary support [2, 18, 21-25]. Admission to the intensive care unit is approximately required for 1-4% of those hospitalized children [26-30]. Mortality of RSV infection in high-income countries is generally rare [31, 32]. The extent of the worldwide burden of RSV is enormous with

33.1 million (21.6-50.3) annual lower respiratory tract infections, resulting in 3.2 million (2.7-3.8) hospitalizations and causing 59.600 (48.000-74.500) in-hospital deaths in children younger than five years [33].

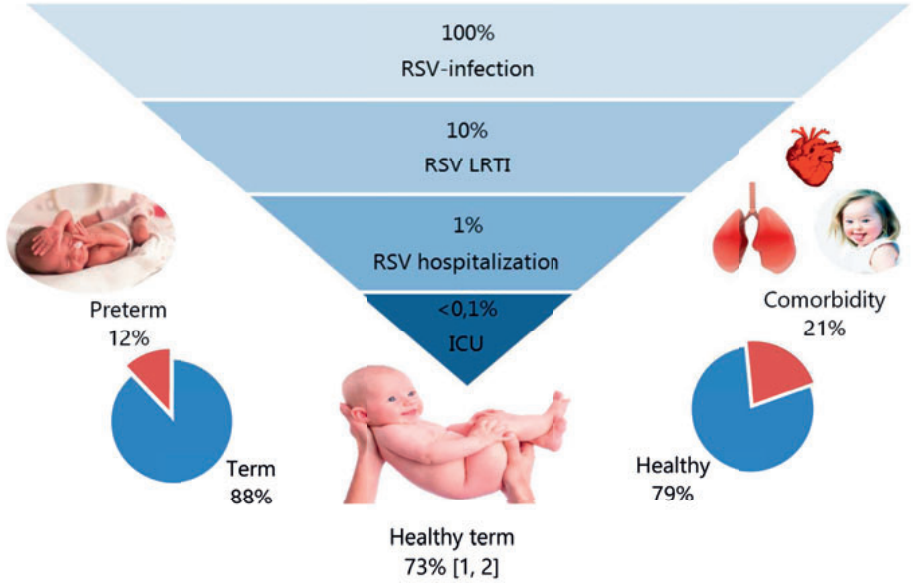

Figure 1. RSV epidemiology in childhood in healthy term born infants and composition of hospitalized children (displayed on sides of the pyramid).

The pyramid displayed in Figure 1 is based on the epidemiology in healthy term born children in high-income countries but can look very different for certain high-risk groups. High-risk groups that more frequently experience severe RSV infection are those that are born prematurely [34], have congenital heart disease [35], congenital lung disease [36], and those with severe underlying medical conditions such as Down syndrome [37]. In these groups RSV hospitalization rates can be up to 35 times higher compared to term born infants. Up to 53% of high risk children that are hospitalized may need intensive care admission [34-37]. Despite the higher hospitalization rates in these groups, most of the children that are hospitalized are healthy term born children, as shown in Figure 1. While in high-income countries supportive care by means of intensive care units and mechanical respiratory support is available, this is often not the case in low-income countries. This lack of available supportive care causes 99% of the worldwide deaths due to RSV to occur in developing countries even though risk factors such as prematurity

and comorbidity are less common in those that die of RSV compared to those that die in high income countries [38, 39].

Prediction of severe RSV infection

Besides prematurity and certain comorbidities, there are many factors that influence the risk of severe RSV infection (requiring hospitalization) in early life. To date, several risk prediction models have been published aiming to predict severe RSV infection in early life [18, 40-46]. However, none are used in clinical practice. This is likely caused by moderate discriminative abilities and a lack of useful application in the absence of (preventive) treatment. Many more individual risk factors have been identified, including the presence of older siblings in the household, being young (<6 months) at the start of the RSV season, male sex, maternal smoking during pregnancy, birth weight and atopic predisposition [47, 48]. Breastfeeding showed to be protective against RSV infection. This may be caused by transfer of specific anti-RSV maternal antibodies [49] in addition to other components that are also likely to play a role since breastfeeding showed to be protective for all-cause (respiratory) infection [50]. The presence of siblings in the household is a major risk factor for RSV infection, especially the ones that visit day-care or primary school where viral circulation is high. Household transmission studies have shown that the older sibling is most often the one that introduces RSV in the family [51-53]. Being young is also a major risk factor for RSV hospitalization, as shown convincingly in two landmark papers of Caroline Breese Hall that indicate that children in the first six months of life have the highest risk of hospitalization due to RSV infection [1, 54]. Physiologically, the average diameter of a respiratory bronchiole of a 2–4 month old infant approximates 120 μm compared to 250 μm for an adult respiratory bronchiole, thus greatly increasing the potential impact of obstruction on the infant bronchiolar lumen during virus-induced inflammation [55]. Additionally, while the child's own natural immunity against RSV has not yet been developed, the protective maternal antibodies obtained via transplacental transfer wane causing a lack of protection during the first few months after birth [17, 56, 57]. Obviously, being young at the start of the RSV season only enhances these effects since you combine the increased vulnerability with an increased risk of exposure to RSV. Male sex has been shown to result in more severe RSV disease in epidemiological studies despite a similar incidence compared to female sex [58]. Underlying causes for these differences are still largely unresolved although part may be explained by the shorter and narrower airways that boys have compared to girls in early childhood [58, 59] These anatomical differences may predispose boys to develop bronchial obstruction upon

RSV infection which results in a more severe course of disease [59]. Maternal smoking during pregnancy may have detrimental effects on lung growth and development by restriction of nutrition because of vascular damage that results in placental insufficiency [60]. Underdeveloped lungs may predispose children to experience more severe disease upon respiratory infection. Both being small for gestational age as well as having a higher birthweight seem to influence the risk of RSV hospitalization [18, 40, 61]. Low birth weight is shown to be associated with decreased lung function [62] but it can also represent a confounding factor in the association of prematurity and RSV infection or the abovementioned placental insufficiency and RSV infection. Last, atopic disease in the parents may predispose children to airway hypersensitivity what could increase the risk of more severe disease due to respiratory infection [63]. This brings us to the association between RSV and long term respiratory sequelae.

RSV and asthma

RSV bronchiolitis is in the majority of cases associated with wheezing and cough which normally can last up to several weeks after acute infection [54, 64]. A subset of children develops recurrent wheezing and even asthma is associated with early life RSV infection [65]. Longitudinal cohort studies have found that children with severe RSV infection may experience recurrent wheeze in the first years of life [63, 66-68], at school-age [21, 69-77] and even up to early adulthood [78-81]. Although the association between severe RSV infection and long term respiratory morbidity and asthma is obvious, it remains unclear whether RSV actually causes development of asthma or whether children with a predisposition for asthma experience more often severe RSV infections as a manifestation of this predisposition. Studies that assessed lung function shortly after birth showed that a decreased lung function actually precedes onset of respiratory infections or asthmatic symptoms and is predictive for the development of severe respiratory infection as well as post-RSV wheeze, and asthma [82, 83]. Two placebo-controlled randomized trials of RSV-prophylaxis have assessed recurrent wheeze and asthma development [20, 84, 85]. Although the MAKI trial found a decrease in the number of wheezing days in the first year of life and a lower incidence of parent-reported asthma at age six, no effect of RSV-prophylaxis was found on lung function or doctor's diagnosed asthma [84, 85]. In the motavizumab trial, no effect of RSV-prophylaxis was observed on the incidence of medically-attended wheeze in the first three years of life [20]. While the observational studies are potentially biased by selection, recall and confounding bias, the RCT's have flaws as well since the MAKI trial was unblinded for parents after the first year of life and

both studies were probably underpowered to actually conclude a statistical significant difference in development of recurrent wheeze and asthma [86]. Unfortunately there are more challenges in the investigation of the relation between RSV and asthma. Atopy may confound this complicated relation since children with atopic predisposition are at increased risk for both asthma as well as severe RSV infection. Another problematic feature in comparison of these studies is how asthma is defined. Uniform definitions of childhood asthma are lacking which is why various (up to sixty different!) asthma outcomes are used in literature [87]. It is unknown to what extent these definitions can validly be compared.

RSV in older adults

Although the majority of people experience their first RSV infection before the age of two, no long-lasting immunity after natural infection is acquired, making individuals prone to reinfection throughout their lives [13, 88]. RSV infections in adulthood are often milder than primary childhood infections, but can still cause severe respiratory disease [89, 90]. This is probably best illustrated by the fact that the overwhelming majority of RSV mortality in industrialized countries occurs in those that are above 65 years of age and not in babies [33, 91]. RSV disease in the adult population is mostly studied in hospitalized patients and nursing home residents showing that the most vulnerable adults for severe RSV infection are those who are older, have an immunodeficiency or underlying cardiopulmonary disease [89, 90, 92]. The disease burden in these patients has been shown to be similar to that of non-pandemic influenza, and can result in lower respiratory tract infection, respiratory failure and even death [89, 92]. Although we are increasingly aware of RSV infection in older adults in medical settings, we still know surprisingly little about RSV-related disease in the general population. Two previous cohort studies in older adults living in the community, so-called community-dwelling older adults, indicated an overall annual incidence of RSV infection of 3-7% in generally healthy older adults [89, 93]. However, both studies are over 15 years old and only the study by Falsey and colleagues [89] used serology and PCR on nasopharyngeal swabs to confirm RSV infection, while Nicholson and colleagues [93] used serology alone. Moreover, both studies did not observe any RSV-related hospitalizations in the healthy community population but were limited by sample size to firmly conclude a lack of severe disease. Therefore, the exact burden of RSV in older adults in the general population is still poorly defined.

Prevention and treatment of RSV infection

Preventive strategies of RSV infection include passive and active immunization including active maternal immunization of pregnant women to protect their newborn child. Direct treatment can be performed using antivirals at the moment of acute infection. As aforementioned, currently only passive immunization with palivizumab or motavizumab is proven to be protective of which only palivizumab is licensed. However, palivizumab is unavailable for most children and parts of the world due to its high costs. Nevertheless, many potential vaccines are currently in clinical trials of which some have already entered Phase three [12]. While Ribavirin is registered for acute treatment of RSV, the moderate effectivity, high costs and significant side effects limit its applicability [94]. Multiple antiviral therapeutics are currently being developed and tested in clinical trials [95]. With new therapeutics on the horizon, it is important that we have a thorough understanding of RSV epidemiology and disease burden to determine which groups would benefit most from these therapeutics and how preventive strategies should be deployed once they become available.

Aims and outlines of this thesis

This thesis aims to better understand disease incidence, severity, risk factors and general epidemiology of RSV infection in populations at both ends of the age spectrum. We work our way up from birth to school-age after which we jump to RSV infection in those with older age.

Specific research questions addressed in this thesis are:

Chapter 2. Can we improve prediction of RSV hospitalization in the first year of life?

Chapter 3. How does atopy define the association between RSV and the risk of recurrent wheeze or asthmatic symptoms?

Chapter 4. How should we define asthma in future (RSV) studies?

Chapter 5. What is the burden of RSV infection in older adults living in the community?

Chapter 6. Does exposure to children increase the risk of respiratory infection in older adults?

Chapter 7. Do the WHO case definitions capture the burden of RSV in older adults?

Part I:RSV in childhood

Part one describes two studies from the RISK birth cohort about RSV prediction (Chapter 2) and long term sequelae of RSV (Chapter 3) as well as one study from the WHISTLER

birth cohort about the definition of asthma (Chapter 4). Many prediction models are published to determine the risk of RSV infection in early life. However, none are routinely used in clinical practice. In **Chapter 2** we aimed to improve a previously defined Dutch prediction model published by Blanken and colleagues [46]. The model was updated and internally validated in over 4000 children to increase performance. We also build a simple scoring system to aid doctors in determining the risk of severe RSV infection at birth in late preterm infants. In **Chapter 3** we followed the children from the RISK birth cohort up to the age of six to investigate the role of atopy in the association between early life RSV infection and development of asthma. Although asthma is commonly diagnosed in young children, a standardized definition of asthma is lacking. Outcome definitions of asthma are used interchangeably in literature although this is perhaps not justified. In **Chapter 4** we have compared agreement between multiple commonly used definitions of asthma based on parental report, doctor's diagnosis and lung function assessed at the ages of five and eight years old to determine agreement between asthma definitions.

Part II: RSV in older adults

In part two we described three studies that originated from the RESCEU older adults cohort study. In preparation for potential future vaccine introduction, this European study in community-dwelling older adults aged sixty years and above was performed to determine the burden of RSV in this population. While the burden of RSV in older adults in the hospital setting is evident, less is known about the incidence and clinical severity of RSV infection in the general older adult population. In **Chapter 5** we present the results of this large European cohort study. Secondly, while for young families we know that school-aged children are often the ones that introduce RSV in the family, we are largely unaware of their role in causing respiratory disease in the older adult population. In **Chapter 6** we have looked at how contact with young children defines the risk of respiratory infection and how much of respiratory disease can be attributed to these contacts. **Chapter 7** discusses the surveillance for RSV. Worldwide surveillance for respiratory disease takes place to monitor epidemics and aid to provide information for public health authorities. Case definitions have been formulated by the world health organization (WHO) to standardize estimates of respiratory disease. However, we do not know how well these case definitions capture RSV in older adults. We determined the performance of existing and alternative RSV specific case definitions to capture RSV infection in older adults.

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PART I
RSV in childhood

CHAPTER 2

Prediction model of RSV-hospitalization following prematurity; validation and updating study.

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ABSTRACT

Background

New vaccines and RSV therapeutics have been developed in the past decade. With approval of these new pharmaceuticals on the horizon, new challenges lie ahead in selecting the appropriate target population. We aimed to improve a previously published prediction model for prediction of RSV-hospitalization within the first year of life.

Methods

Two consecutive prospective multicenter birth cohort studies were performed from June 2008 until February 2015. The first cohort (RISK-I, n=2524, 2008-2011) was used to update the existing model. The updated model was subsequently validated in the RISK-II cohort (n=1564, 2011-2015). We used the TRIPOD criteria for transparent reporting.

Results

181 infants (n=127 in RISK-I, n=54 in RISK-II) were hospitalized for RSV within their first year of life. The updated model included the following predictors; day care attendance and/or siblings (OR: 5.3; 95% CI 2.8-10.1), birth between Aug. 14th and Dec. 1st (OR: 2.4; 1.8-3.2), neonatal respiratory support (OR 2.2; 1.6-3.0), breastfeeding ≤4 months (OR 1.6; 1.2-2.2) and maternal atopic constitution (OR 1.5; 1.1-2.1). The updated models' discrimination was superior to the original model in the RISK-II cohort (AUROC 0.72 95% CI 0.65-0.78 versus AUROC 0.66, 95% CI 0.60-0.73, respectively). The updated model was translated into a simple nomogram to be able to distinguish infants with high versus low risk of RSV-hospitalization.

Conclusion

We developed and validated a clinical prediction model to be able to predict RSV-hospitalization in preterm infants born within 32-35 weeks gestational age. A simple nomogram was developed to target RSV therapeutics to those children who will benefit the most.

INTRODUCTION

New RSV (respiratory syncytial virus) pharmaceuticals have been developed in the past decade and are currently evaluated in clinical trials [1, 2]. With approval of new RSV pharmaceuticals on the horizon, challenges lie ahead in the identification of suitable target populations for these new therapeutics. Prematurity is a known risk factor for RSV-related admission with RSV-hospitalization rates within the first year of life ranging from 4-6% reported in late preterm infants, compared to 1-3% for term born infants [3-9]. This makes RSV the leading cause of acute respiratory tract infection in late premature infants born within 32-35 weeks gestational age (wGA) [8, 10-14]. Several RSV-hospitalization prediction models, including by ourselves, for late preterm infants (32-35 wGA) have been developed so far [3, 5, 7, 15-17]. We previously published a clinical prediction model for RSV-hospitalization in the first year of life in late preterm infants (RISK-I) [3]. The current RISK-II study aimed to improve the performance of the original model and focus on making it easy to obtain a personalized prediction for each individual infant at birth. The high risk patients, who will benefit the most of RSV therapeutics, can be identified for targeted prevention strategies. Low risk patients with hospitalization rates comparable to term born infants are not in need for any specific intervention [6, 9].

METHODS

The RISK study is a prospective multicenter birth cohort study. Otherwise healthy children born within 32 weeks and 1 day to 35 weeks and 6 days of gestational age (referred to as 32-35 wGA) were included during admission at birth at the paediatric departments of the participating hospitals. The Dutch RSV Neonatal network consists of one university hospital and 40 regional hospitals distributed throughout the Netherlands. Children with gross congenital abnormalities (e.g. Down syndrome), and those who received palivizumab for any reason were excluded from the current data analysis.

Original model and development study (RISK-I)

The original model by Blanken et al. [3] was created in the RISK-I study, using infants born from June 2008 until February 2011. Multivariate logistic regression analysis was used to update an existing prediction model [16] in a derivation cohort (n = 1227, 2008-2009) and subsequently validate the updated model in a second prospective cohort (n = 1194, 2009-2011). The original model is shown in Table S1 of the supplemental data.

Validation and updating study (RISK-II)

For the current RISK-II study, two prospective cohorts (RISK-I, RISK-II) were created by a non-random split in time (1st of February 2011). The RISK-I cohort (n=2524, 2008-2011), was used to update the original RISK model. The second prospective cohort (RISK-II, n=1564, 2011-2015) was used for validation of the updated prediction model. The same predictors derived from both the medical records and a parental baseline questionnaire were used, as previously described by Blanken et al. [3] High parental education was defined as at least one of the parents having a university of applied sciences degree. Composite variables were created for day care attendance and/or siblings and for parental atopic constitution, defined as presence of eczema, asthma or hay fever. Dichotomous variables were created for predicted duration of breastfeeding and birth in relation to the RSV season, of which the start was defined at the first week of October for the Netherlands [18]. Parents who could not be contacted by phone after the first year were sent a letter or e-mail for additional contact information. Incorrect contact data were checked in the hospital of admittance at birth followed by contacting the child's general practitioner. If no additional or updated contact data were available, patients were considered lost to follow-up. Multiple imputation was performed to correct for any missing data in the predictor variables used for updating the model.

Outcome

Like the development study by Blanken et al. [3], parents were contacted by telephone after one year for a second questionnaire about occurrence of hospitalization for respiratory disease in the first year of life. Reported hospitalization for respiratory disease within the first year of life was verified in the attended hospital. RSV bronchiolitis hospitalization within the first year of life, the main study endpoint, was defined as hospitalization for respiratory tract infection with proven RSV infection as determined by routine practice laboratory testing in the participating hospitals.

Statistical analysis

At first the original model was validated in the RISK-I cohort. Subsequently, updating of the intercept was performed [19]. Second, based on the moderate discriminative performance of the original model despite adjustment of the intercept, the entire model was refitted by excluding non-significant variables from the model using logistic regression analysis. The updated model was built from the backbone of remaining statistical significant predictors in a stepwise forward selection process including additional predictors. The individual discriminative contribution of included predictors was assessed by the increase in the area under the receiving operator curve (AUROC) and statistical significance ($p<0.05$) for the included variables [20, 21]. Optimism of the updated model was corrected by bootstrapping in the RISK-I cohort [19]. Third, the updated model was validated in the RISK-II cohort. Fourth, due to power issues because of the relatively few amount of cases in the RISK-II cohort, the updated model was once more recalibrated in the total study population (RISK-I and RISK-II combined) in order to obtain the most robust model. Recalibration was performed by adjusting the models' intercept and regression coefficients for optimism in the total study population through a second bootstrapping exercise.

Logistic regression analysis of the updated model provided individualized predicted probabilities for RSV-hospitalization for each patient. A nomogram was created using the adjusted regression coefficients (each multiplied by 2,5) to create a point score which could be linked to the accompanied predicted probability for RSV-hospitalization. Data analysis was carried out using SPSS IBM 22.0 (SPSS Inc, Chicago, Ill).

Role of funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Ethics statement

The RISK study protocol was approved by the Institutional Review Board of the University Medical Center Utrecht and subsequently approved by Institutional Review Boards of all participating hospitals. All parents provided written informed consent for study participation. The study was conducted in compliance with the Declaration of Helsinki and the standards of Good Clinical Practice.

Transparency

This report was written using the TRIPOD statement, a checklist specifically designed for reporting multivariable prediction models. Transparent reporting improves reproducibility and objective judgement of the presented model [22]. This checklist can be found in the supplemental data [Table S5].

RESULTS

Participants

The RISK-I cohort included 2840 patients who completed the baseline questionnaire. After exclusion of patients lost to follow-up, non-RSV related death within the first year, children receiving palivizumab, Down syndrome and other major congenital abnormalities, 2524 patients were included for analysis [Fig. 1]. Hospitalization for respiratory tract infection occurred in 185 (7,3%) children. In these hospitalized patients, RSV test results were positive in 127 cases (5,0%), negative in 42 cases (1,7%) and not performed in 16 cases (0,6%) [Table S2].

The RISK-II cohort included 1921 patients who completed the baseline questionnaire. After exclusion of patients lost to follow-up, non-RSV related death within the first year, children receiving palivizumab, Down syndrome and other major congenital abnormalities, 1564 patients were included for analysis. Hospitalization for respiratory tract infection occurred in 120 (7,7%) children. In these hospitalized patients, RSV test results were positive in 54 cases (3,5%), negative in 27 cases (1,7%) and not performed in 39 cases (2,5%) [Table S2].

Altogether, 4761 patients were included by completing the baseline questionnaire. Follow-up was completed in 4293 patients. 468 (9.8%) Patients were lost to follow-up [Figure 1]. Baseline characteristics for patients lost to follow-up showed that these patients were more likely to be single birth females, of which the parents had more atopic constitution (hay fever and eczema) with more maternal smoking during pregnancy, less predicted day care attendance and an lower average educational level [Table S3]. Baseline characteristics for the RISK-I and RISK-II cohort were comparable except for maternal hay fever, siblings and neonatal respiratory support [Table 1]. Distribution of potential predictors in the RISK-I and RISK-II cohort is shown in Table S4.

Table 1. Distribution of patients characteristics

	RISK-I cohort Jun 2008 - Jan 2011 (n= 2524)		RISK-II cohort Feb 2011 - Feb 2015 (n=1564)	
Clinical data	N (%)	Missing	N (%)	Missing
Gender male	1403 (55,6%)	-	853 (54,5%)	-
Gestational age (wk+ days)	34+2	-	34+2	-
Birth weight (grams) (mean(SD))	2218 (439)	-	2237 (454)	-
Multiple birth	879 (34,8%)	9 (0,4%)	504 (32,2%)	4 (0,3%)
Caesarean section	888 (35,2%)	15 (0,6%)	576 (36,8%)	6 (0,4%)
Neonatal respiratory support [a]	512 (20,3%)	-	405 (25,9%)*	-
Mechanical ventilation	85 (3,4%)	-	38 (2,4%)	-
Birth from August 14th - December 1st	836 (33,1%)	-	488 (31,2%)	-
Parental questionnaire				
Breastfeeding[c]	1889 (74,8%)	14 (0,6%)	1170 (74,8%)	4 (0,3%)
Breastfeeding ≤4 months or not [b]	1315 (52,1%)	14 (0,6%)	808 (51,7%)	4 (0,3%)
Presence of siblings	1006 (39,9%)	12 (0,5%)	548 (35%) *	2 (0,1%)
Planned day care attendance	1497 (59,3%)	19 (0,8%)	904 (57,8%)	5 (0,3%)
Day care attendance/ siblings [c]	1954 (77,4%)	15 (0,6%)	1154 (73,8%) *	5 (0,3%)
Maternal atopic constitution [d]	864 (34,2%)	434 (17,2%)	640 (40,9%) *	9 (0,6%)
Maternal hay fever	436 (17,3%)	567 (22,5%)	437 (27,9%) *	3 (0,2%)
Maternal asthma	300 (11,9%)	7 (0,3%)	159 (10,2%)	2 (0,1%)
Maternal eczema	420 (16,6%)	17(0,7%)	280 (17,9%)	7 (0,4%)
Fur bearing pets	1177 (46,6%)	16 (0,6%)	746 (47,7%)	15 (1%)
Maternal smoking during pregnancy	313 (12,4%)	2 (0,1%)	184 (11,8%)	2 (0,1%)
High parental educational level [e]	1441 (57,1%)	40 (1,6%)	944 (60,4%)	23 (1,5%)

* Baseline difference in between cohorts P<0.05. [a] Oxygen/nasal mask/CPAP and/or mechanical ventilation. [b] predicted, either exclusive/mixed with formula feeding. [c] composite of presence of siblings and/or planned day care attendance. [d] hay fever/asthma and/or eczema. [e] One parent completed at least a university of applied sciences.

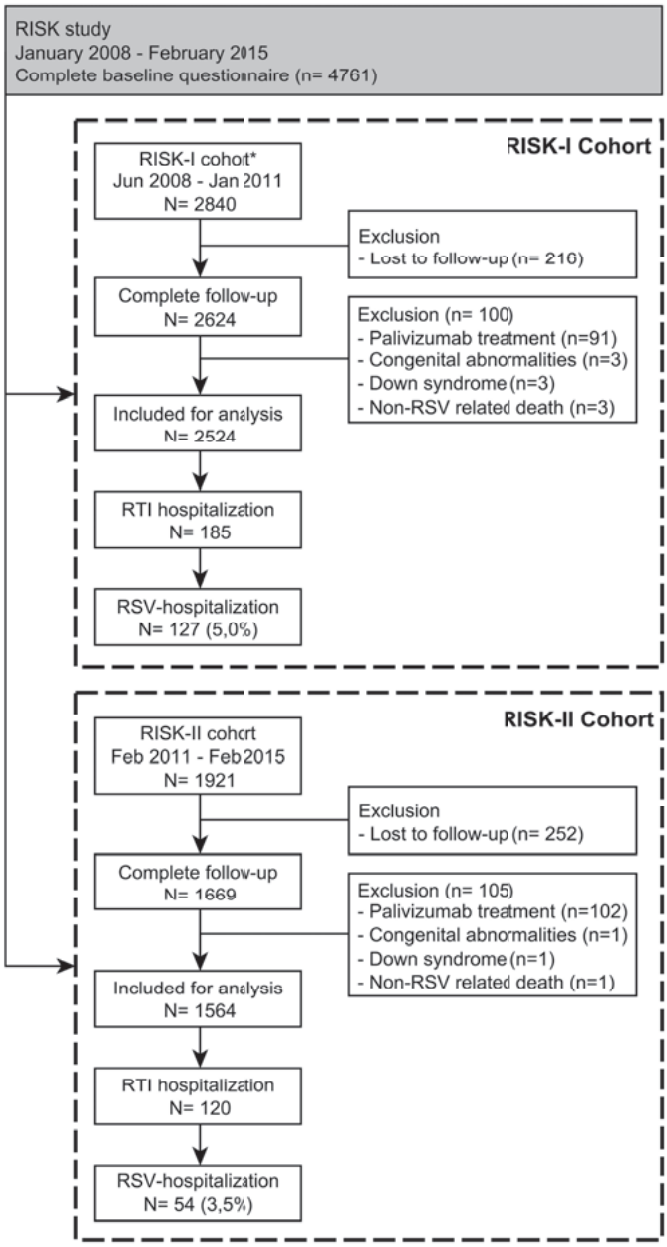

Figure 1. Flowchart of patients recruited into the study. *The RISK-I cohort included the original models' derivation (n = 1227), validation cohort (n = 1194) and additional patients (n = 103) born before the 1st of February who completed follow-up after the original article was written by Blanken et al.[3] Abbreviations: RTI = respiratory tract infection; RSV = respiratory syncytial virus.

Validation of the original model

The original RISK model was validated in the RISK-I cohort. The AUROC of the original model was 0.69 (95% CI 0.65 – 0.74). The calibration plot in the RISK-I cohort showed an intercept of 0.0 slope of 1.0 with average absolute difference in predicted and calibrated probabilities (Eavg) of 0.002. Adjusting the intercept by calculating a correction factor did not affect discrimination (AUROC 0.69 95% CI 0.65 – 0.74) and calibration as expected since the original model was bootstrapped on half the RISK-I cohort in the development study [3].

Updating of the original model

Based on the moderate discriminative performance the entire model was refitted. Of the four variables in the original model, only the variables 'day care attendance and/or siblings' and 'birth from August 14th – December 1st' remained significant contributors to the model in logistic regression analysis. Predicted duration of breastfeeding was adjusted to ≤4 months instead of ≤2. '1st degree family atopy' was further specified to maternal atopic constitution. Lastly, neonatal respiratory support was included [Table 2]. After updating the model, the discrimination (AUROC) in the RISK-I cohort was 0.72 (95% CI 0.67-0.76) before and after bootstrapping [Table 2].

Table 2. Updated RISK model characteristics.

Predictors	Regression coefficient [a]	Odds ratio (95% CI)	P-value
Day care attendance and/or siblings [b]	1.605	5.3 (2.8-10.1)	<0.001
Birth from Aug 14th to Dec 1st	0.858	2.4 (1.8-3.2)	<0.001
Neonatal respiratory support [c]	0.758	2.2 (1.6-3.0)	<0.001
Maternal atopic constitution [d]	0.405	1.5 (1.1-2.1)	0.01
Breastfeeding ≤4 months or not [e]	0.470	1.6 (1.2-2.2)	0.003
Intercept	-5,484		
AUROC (95% CI) RISK-I cohort		0.72 (0.67-0.76)	
AUROC (95% CI) RISK-II cohort		0.72 (0.65-0.78)	
AUROC (95% CI) Total population		0.72 (0.68-0.75)	

[a] Adjusted coefficients after bootstrapping in total study population. [b] Composite variable of presence of siblings and/or planned day care attendance. [c] Oxygen/nasal mask/CPAP and/or mechanical ventilation. [d] Either maternal hay fever, asthma and/or eczema. [e] Predicted.

Validation of the updated model

The updated models' performance subsequently was tested in the RISK-II cohort. Discrimination proved similar (AUROC 0.72, 95% CI 0.65-0.78) and calibration showed a

non-significant Hosmer-Lemeshow test (P= 0.63) indicating a good model fit with the calibration plot showing an intercept of 0.0, slope of 1.0 and Eavg of 0.001 [Figure S1].

Recalibration of the updated model

The final model was obtained by recalibration through a second bootstrapping exercise of the updated model in the total study population (RISK-I + RISK-II). The updated RISK model specifics are shown in Table 2.

Application of the updated model.

In order to maximize the applicability of the presented model, a nomogram was created based on the predicted probabilities, in which the predicted risk for hospitalization for individual patients can be easily assessed [Figure 2]. Individual hospitalization risks vary from 0.4% up to 20% within the first year. The model is able to identify a group at high risk (average risk of 13%) while selecting 11% of the total population (total score of ≥8 in the nomogram. Exploration of low risk infants according to the predicted probabilities identified a group consisting of 34% of the total study population with an average hospitalization risk of 1% within the first year of life (score ≤4 in the nomogram). Both risk categories are presented in Table 3.

Table 3. Risk group characteristics in total population (n= 4088)

Risk category	Predicted hospitalization risk (range)	(n/N)[a]	%[b]	Sensitivity	Specificity	PPV	NPV
Low risk	1% (0.4-2.1%)	18/1373	34%	0.90	0.35	0.06	0.99
High risk	13% (10-20%)	58/401	11%	0.32	0.90	0.13	0.97

Abbreviations: PPV = positive predictive value. NPV = negative predictive value. [a] Number of patients in this risk category; n= cases N= controls. [b] Percentage of the total study population.

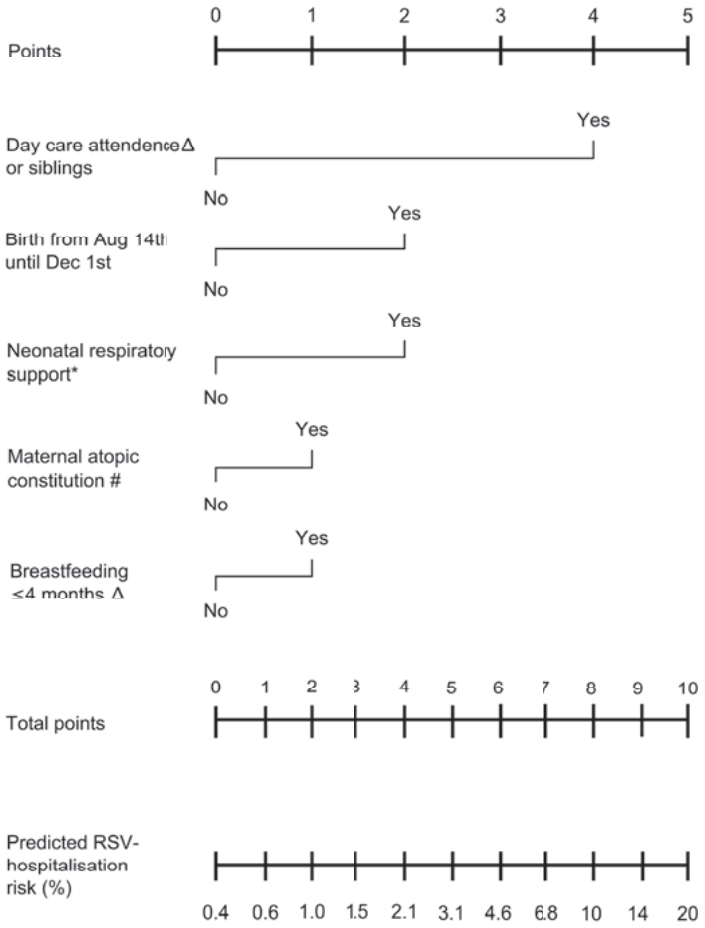

Figure 2. RISK nomogram. ▲Predicted. *Oxygen/nasal mask/CPAP and/or mechanical ventilation. ■ Either maternal hay fever, asthma or eczema. To illustrate the nomograms' use for the individual patient, let us consider an infant born at34 + 3 wGA on the first of November. The infant had a good start and did not need neonatal support. This is the parents' first child and the mother (who is asthmatic) is willing to breastfeed the newborn for at least half a year. She is able to take care of the child so day care attendance is not needed. This adds up to a total of 3 points (birth period and atopic mother), resulting in a predicted risk of 1.5% of RSV-hospitalization for this specific child in the first year of life.

DISCUSSION

We have prospectively validated and updated a prediction model to identify late preterm infants born within 32-35 weeks of gestation at high versus low risk of respiratory syncytial virus hospitalization in the first year of life. The presented model is easy to use in a clinical setting and can inform both parents and medical staff on the individual risk of RSV-hospitalization for newly born preterm infants. With new RSV therapeutics on the horizon, the presented model can be used as a tool to assess the RSV-hospitalization risk in order to target RSV therapeutics to those children who will benefit most.

Several prediction models have been published to assess the hospitalization risk for RSV in late preterm infants [3, 7, 15-17]. Common predictors are presence of siblings, day care attendance and young chronological age in relation to the RSV season. Familial atopic constitution is both noted as a risk factor [3, 16], as well as a protective factor for RSV-hospitalization [17]. Maternal atopic constitution has been associated with an increased severity of rhinovirus-related illness in infants, but the relation with RSV remains poorly understood [23]. The protective effect of breastfeeding proved significant, which is in line with published literature [16, 24-27]. Neonatal respiratory support has not been included in previous prediction models, but has recently been suggested as an independent risk factor for RSV-hospitalization [28]. Increased risk of respiratory infection following neonatal respiratory support can be due to a pre-existent immature lung function that urges the need for respiratory support, or can be caused by damage due to the respiratory support itself. None of the included patients developed bronchopulmonary dysplasia (BPD), therefore we speculate that immature lung function is key in making these infants more susceptible for respiratory infection in the first year of life.

Major strengths of our study include model improvement in two large independent prospective cohorts with a low incidence of palivizumab (<5%). By updating the original model instead of creating an entirely new one, we aimed to preserve rather than discard previous knowledge. The presented prediction model has previously been validated, making the results of the current update, validation and recalibration even more solid. We used TRIPOD criteria for transparent reporting of prediction models in order maximize objective reporting of the presented model.

There are certain limitations to be mentioned. First, there could be an underestimation of the RSV-hospitalization incidence. In the Netherlands, routine testing for RSV tends

to decrease because a positive test does not lead to a different therapeutic approach. Underestimation of the risk of RSV-hospitalization is unlikely to have affected the AUROC of the prediction model, but would result in an underestimation of the positive predictive value. Second, 9.8% of included patients could not be contacted despite extended efforts and were considered lost to follow-up, creating potential selection bias. Third, although overall missing values were scarce, parental hay fever was later added to the parental questionnaire resulting in 567 missing values in the RISK-I cohort. Multiple imputation was performed to minimize bias by missing values. Fourth, RSV incidence was notably lower in the RISK-II cohort. Although mild winters and therefore decreased RSV incidence during the last years of inclusion was noted in the Netherlands[18], a more likely explanation is a decrease in routine RSV testing. The percentage of non-tested patients increased from 9% in the RISK-I cohort to 33% in the RISK-II cohort. The incidence of hospitalizations for respiratory infections in both cohorts was comparable (7,4% versus 7,7% for RISK-I,II respectively).

Future research should focus on the confirmation of validity of the model in an external cohort and evaluate the potential impact of the updated RISK prediction model during implementation in clinical guidelines.

CONCLUSION

We validated and improved an existing prediction model for respiratory syncytial virus hospitalization within the first year of life in late preterm infants born within 32-35 weeks gestational age. This prediction model can aid in the risk assessment at birth for preterm born infants and is sufficiently sensitive to identify both high risk infants for targeted therapy as well as infants not requiring any specific intervention.

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CHAPTER 3

RSV hospitalization in infancy increases the risk of current wheeze at age six in late preterm born children without atopic predisposition

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ABSTRACT

Background

Severe respiratory syncytial virus (RSV) infection during infancy is associated with ongoing respiratory morbidity.

Methods

In a large birth cohort of 2210 healthy preterm infants born at 32–35 weeks of gestation, we aimed to determine the role of atopy in the link between RSV hospitalization and current wheeze at age six. We defined current wheeze as parent-reported wheeze or the use of respiratory medication in the past 12 months. Based on a positive family history of atopic disease, we distinguished between children with and without atopic predisposition.

Results

Six-year follow-up data was obtained in 997/1559 (64%) children of which 102 (10.2%) children had been hospitalized with RSV during infancy. Current wheeze was present in 184/997 (18.6%) children. RSV hospitalization was an independent risk factor for current wheeze in children without atopic predisposition (aOR 4.05 [95% CI 1.22–12.52]) but not in children with this atopic background (aOR 1.50 [95% CI 0.81–2.71]).

Conclusion

This is the largest published birth cohort demonstrating that in late preterm infants, atopic predisposition defines the relationship between RSV hospitalization and current wheeze. Future RSV prevention trials aiming to prevent ongoing respiratory symptoms should be analyzed separately for atopic status.

INTRODUCTION

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection (LRTI) in infants globally and is responsible for a vast burden of disease in early childhood [1-3]. Severe RSV infection is not only responsible for morbidity in early childhood but can also cause ongoing respiratory morbidity up to early adulthood [4-6]. This ongoing respiratory morbidity is characterized by persistent wheezing [4, 7-11], decreased lung function [4, 5, 11], and is suggested to influence the development of asthma [4, 6, 10-12]. However, the association between severe RSV infection and ongoing symptoms seems to decrease with age which raises doubt about whether this respiratory morbidity is permanent or merely transient [13]. Additionally, we lack definitive proof that shows that severe RSV infection is causal in the development of ongoing respiratory morbidity or simply reflects an underlying predisposition for respiratory morbidity caused by other pathophysiological mechanisms such as atopy [14]. While we know that atopic children have an increased risk of developing asthma, we are unaware of the precise interplay between atopy and severe RSV infection in infancy in causing persistent respiratory morbidity. Few studies have investigated this specific relation and results are conflicting [9, 11, 12]. These studies were often underpowered in subgroup analysis which made it difficult to draw robust conclusions. We aimed to determine the role of atopy in the link between severe RSV infection in early childhood in late preterm infants and ongoing respiratory symptoms at school age.

METHODS

Study design

This study is a prospective follow-up study of the RISK study, a multicenter, prospective birth cohort study to investigate risk factors for RSV hospitalization (RSVH) in otherwise healthy late preterm infants of 32^{+1} - 35^{+6} weeks gestational age. The study design and data collection of the RISK study has been explained in detail in previous publications [15, 16]. To summarize, otherwise healthy children born between 32^{+1} to 35^{+6} weeks of gestation were included in 41 participating hospitals in the Netherlands between June 2008 and February 2015. Children with major congenital abnormalities (such as Down syndrome), children who received palivizumab for any reason, and children hospitalized for clinical bronchiolitis without a viral test result were excluded from analysis. In total, 4088 children were included in the RISK study of which 181 (4.4%) were hospitalized in the first year of life with RSV-bronchiolitis [16]. In the current study we prospectively followed up children from this cohort that reached the age of six years, using an online parental questionnaire.

first year follow-up

Clinical data about pregnancy and birth characteristics were obtained from medical records retrieved from the hospital where the child was born. Additionally, parents filled out a questionnaire at birth about risk factors of RSV infection including the presence of siblings, planned day care attendance, atopy in first degree family members and parental smoking. After the first year of life a second parental questionnaire was completed. This questionnaire recorded information about hospitalization for respiratory infection, presence of wheezing, the use of respiratory medicine, breastfeeding, day care attendance and eczema. If hospitalization for respiratory infection was indicated by the parents at 1 year follow-up, we retrospectively verified whether RSV-infection was the cause of hospitalization in the medical records of the attended hospital.

Six year follow-up

Follow-up at six years of age was performed using an online parental questionnaire. Data was collected about asthma, wheezing and the use of respiratory medication. This questionnaire was based on the standardized core questions from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [17]. The questionnaire was completed in an online survey tool (NetQ healthcare software https://www.netqhealthcare.nl/en). Participants received an invite for follow-up by email. and were

reminded to participate once more after two weeks if they had not responded. To increase statistical power, we enriched the cohort with active telephonic follow-up of additional RSVH cases from the non-responders.

Definitions

Severe RSV infection was defined as hospitalization for respiratory tract infection with proven RSV infection as determined by routine practice laboratory testing in the participating hospitals [15, 16]. Our primary outcome was current wheeze at the age of six years. We defined current wheeze as parent-reported wheeze or the use of respiratory medication, or both, within the past twelve months. Parent-reported wheeze was defined as at least one reported episode of wheeze in the past 12 months, whereas reported use of respiratory medication was defined as the use of inhaled respiratory medication (either inhaled steroids or beta-mimetics) at least once in the past 12 months. Atopic predisposition was defined as a positive history of atopic disease (asthma, eczema or hay fever) in at least one of the parents.

Statistical analysis

First, crude odds ratios for the association between RSVH and current wheeze were calculated for children with complete follow-up data using logistic regression analysis. Second, we compared baseline characteristics between the response and non-response group using the Student's t-test and Mann-Whitney U test for continuous variables and the $\chi 2$ test or Fisher Exact test for discrete variables with a significance level of <0.05. Statistically significant variables that indicated non-response or 'missingness' were included in the logistic regression model in order to adjust for this non-response. Third, we adjusted for potential confounding bias by including potential confounders from literature to the model. We performed an extensive literature search to identify risk factors for RSV and asthma that could potentially confound the results [supplemental table S1]. Fourth, we stratified the results by atopic predisposition in order to obtain estimates for children with and without atopic predisposition.

Last, we performed a sensitivity analysis in which the missing outcome data was imputed for children without six year follow-up. We used multiple imputation with 100 iterations per imputed dataset to obtain 30 imputed datasets. Analyses were performed on the 30 imputed datasets and Rubin's rules were applied to obtain overall estimates. All analyses were performed using R version 3.1.1 for Windows and multiple imputation was performed with the mice package.

Ethics statement

The RISK study was reviewed and approved by the Institutional Review Board of the University Medical Centre Utrecht and subsequently approved by Institutional Review Boards of all participating hospitals. All parents provided written informed consent for screening of hospital records. The study was conducted in compliance with the Declaration of Helsinki and the standards of Good Clinical Practice. This manuscript was written according to the guidelines from the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [18].

RESULTS

Participants

2307 children from the RISK birth cohort were eligible for follow-up at age six because they were born between June 2008 and April 2011. We excluded 78 children receiving palivizumab, 6 children with major congenital abnormalities, and 13 patients hospitalized with clinical bronchiolitis but in whom no viral testing was performed). The remaining 2210 children were contacted for participation in the online survey. 651 (29%) of the participants could not be reached because of incorrect contact details. 1559 participants received the invitation, of which 961 (62%) completed the online questionnaire. With active telephonic follow-up we included 36 additional RSVH cases from the non-response group, resulting in a total of 997 children with six year follow-up data [Fig. 1]. Hospitalization because of RSV infection occurred in 102 children during their first year of life. Baseline characteristics of children with and without six year follow-up are shown in Table 1. Various differences we seen between children with and without complete follow-up [Table 1]. These differences remained significant when the 36 cases which were included by active follow-up were analyzed in their original group (data not shown).

Table 1. Baseline characteristics stratified by response to six year follow-up.

	Response (N=997)	No response (N=1213)	P-value [a]
Birth characteristics			
Gender male	561 / 997 (56.3%)	657 / 1213 (54.2%)	0.3
wGA (weeks+days)	34+2	34+2	0.4
Birth weight (in grams (SD))	2219 (434)	2222 (444)	0.9
Multiple birth	317 / 995 (31.9%)	453 / 1207 (37.5%)	0.005
Maternal asthma	101 / 995 (10.2%)	164 / 1209 (13.6%)	0.01
Paternal asthma	72 / 995 (7.2%)	162 / 1209 (13.4%)	<0.001
Maternal eczema	151 / 992 (15.2%)	220 / 1205 (18.3%)	0.06
Paternal eczema	113 / 992 (11.4%)	176 / 1205 (14.6%)	0.03
Maternal hay fever	173 / 762 [b] (22.7%)	238 / 986 (24.1%)	0.5
Paternal hay fever	149 / 762 [b] (19.6%)	231 / 986 (23.4%)	0.08
Maternal smoking during pregnancy	107 / 997 (10.7%)	161 / 1212 (13.2%)	0.07
High educational level mother [c]	508 / 993 (51.2%)	497 / 1206 (41.2%)	<0.001
High educational level father [c]	461 / 985 (46.8%)	478 / 1195 (40.0%)	0.001
Dutch nationality mother	907 / 997 (91.0%)	1060 / 1213 (87.4%)	0.01
Dutch nationality father	902 / 997 (90.5%)	1052 / 1213 (86.7%)	0.01
Siblings (at least one)	375 / 996 (37.7%)	480 / 1206 (39.8%)	0.3
Follow-up at one year of age			
RSVH [d]	102 / 997 (10.2%)	19 / 1213 (1.6%)	<0.001
Wheeze	297 / 995 (29.8%)	340 / 1207 (28.2%)	0.4
Respiratory medicine use (at least once)	255 / 989 (25.8%)	289 / 1200 (24.1%)	0.4
Smoking mother	148 / 995 (14.9%)	240 / 1210 (19.8%)	0.002
Smoking father	256 / 993 (25.8%)	365 / 1209 (30.2%)	0.02
Day-care attendance	608 / 998 (61.0%)	628 / 1212 (51.8%)	<0.001
Eczema	286 / 994 (28.8%)	369 / 1209 (30.5%)	0.4
Breastfed [e]	754 / 997 (75.6%)	863 / 1210 (71.3%)	0.02

Data are presented as: n / total number of participants with data (%), unless otherwise specified. Abbreviations: wGA = weeks gestational age; SD = standard deviation; RSVH = RSV hospitalization; N/A = Not applicable. [a] P-value based on univariate comparison between response and non-response group. [b] Parental hay fever was later added to the parental questionnaire resulting in a higher number of missing values. [c] Educational level was dichotomized by using the arbitrary cut-off level of at least obtaining a institute for Higher Profession Education and Training degree (called HBO in the Netherlands). [d] Numbers after enrichment by active follow-up of RSVH cases from the non-response group. The original distribution before enrichment was 66/961 (6,9%) vs 55/1249 (4,4%) (p=0.01). [e] Received breastfeeding for at least one week after birth.

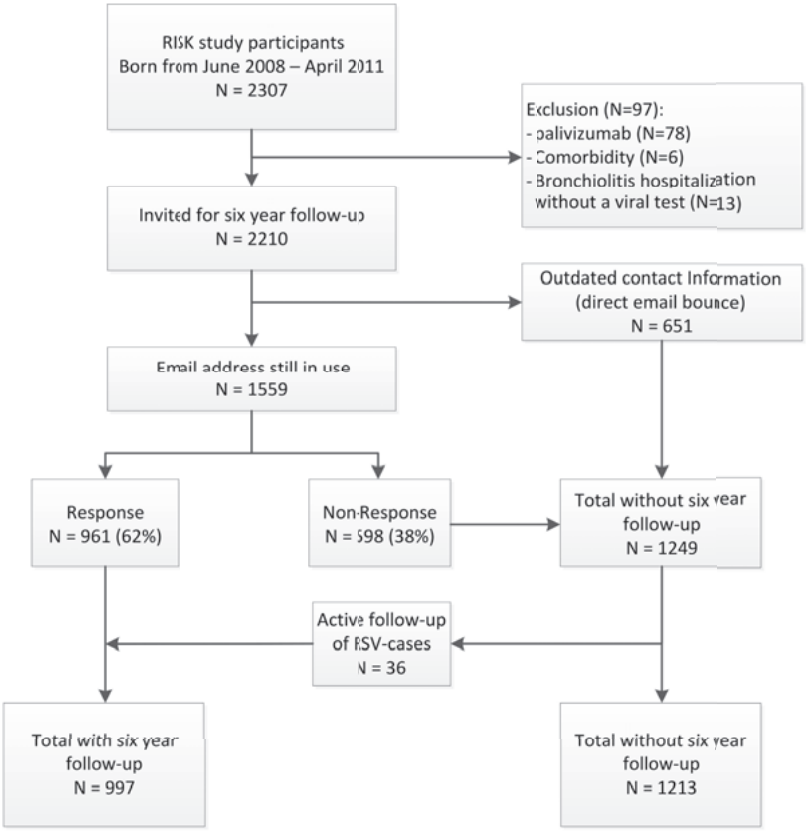

Figure 1. Flowchart of study participants

Current wheeze

At six years of age wheeze was reported in 119/997 (12%) children and the use of respiratory medication was reported in 159/988 (16%) children. In 9 children data about the use of respiratory medication was missing resulting in 8 undetermined values for the composite endpoint of current wheeze (1 indicated wheezing in past 12 months and was classified as having current wheeze for the composite endpoint). The composite endpoint current wheeze was present in 184 of 989 (18.6%) children with available data.

Atopic predisposition

Baseline data about atopic predisposition was available in 828 children. Atopic predisposition was present in 506/828 (61%) of the children. This reflects that at least one of the parents of the child has indicated having eczema, hay fever or asthma. When we stratified for atopic predisposition, we saw that 118/506 (23.3%) of the children with

atopic predisposition had current wheeze compared to 40/322 (12.4%) of children without atopic predisposition

RSV, atopic predisposition and current wheeze

At the age of six, 28/101 (27.7%) of RSVH cases had current wheeze compared to 156/888 (17.6%) children who were not hospitalized [Table 2]. The unadjusted odds ratio for RSVH and current wheeze at six years of age was 1.80 (95% CI 1.11-2.85). This association was stable after subsequent adjustment for non-response (adjusted OR 1.97; 95% CI 1.11-3.39), and after adjustment for confounding (aOR 1.89; 95% CI 1.06-3.32) [Table 2].

After stratification for atopic predisposition the association between RSVH and current wheeze was statistically significant in children without atopic predisposition (aOR 4.05; 95% CI 1.22-12.52) but was not significant in children with atopic predisposition (aOR 1.50; 95% CI 0.81-2.71) [Table 2]. When sensitivity analysis was performed in the total study population with imputation of missing data the association between RSVH and current wheeze was confirmed only in children without atopic predisposition [Table 3].

Table 2. The risk of current wheeze at age six stratified by atopic predisposition

Outcome	RSVH	No RSVH	OR (95% CI)	aOR [b] (95% CI)	aOR [c] (95% CI)
Current wheeze (Overall)	28/101 (27.7%)	156/888 (17.6%)	1.80 (1.11-2.85)	1.97 (1.11-3.39)	1.89 (1.06-3.32)
Current wheeze (Atopic predisposition)	19/62[a] (30.6%)	99/444 [a] (22.3%)	1.54 (0.84-2.73)	1.54 (0.84-2.76)	1.50 (0.81-2.71)
Current wheeze (No atopic predisposition)	6/21 [a] (28.6%)	34/301[a] (11.3%)	3.14 (1.06-8.31)	3.39 (1.12-9.24)	4.05 (1.22-12.52)

Abbreviations: RSVH = RSV hospitalization; OR = odds ratio; aOR = adjusted odds ratio. [a] 169 participants (19 RSVH cases, 150 participants without RSV infection) had missing data on atopic predisposition resulting in 828 participants to be analyzed when we stratified for atopic predisposition (506 atopic, 322 nonatopic). [b]Adjusted odds ratios were corrected for variables of missingness (multiple birth, parental smoking, parental asthma, parental eczema, parental educational level, non-Dutch nationality, day-care attendance and breastfeeding), but not for confounding. [c] Adjusted odds ratios were corrected for both variables of missingness and potential confounders (male gender, presence of siblings, birth weight, day care attendance, smoke exposure, parental atopic constitution, breastfeeding and educational level of the parents).

Table 3. Sensitivity analysis of the risk of current wheeze at age six stratified by atopic predisposition

Outcome	RSVH	No RSVH	OR (95% CI)	aOR (95% CI) [a]
Current wheeze (Overall)	28%	20%	1.54 (0.96-2.47)	1.53 (0.93-2.53)
Current wheeze (Atopic predisposition)	30%	25%	1.31 (0.74-2.30)	1.32 (0.85-2.04)
Current wheeze (No atopic predisposition)	22%	13%	1.92 (0.81-4.56)	1.88 (1.07-3.30)

Data represent the average percentages of the 30 imputed datasets. Abbreviations: RSVH = RSV hospitalization; OR = odds ratio; aOR= adjusted odds ratio. [a] Adjusted odd ratios were corrected for potential confounders (male gender, breastfeeding, siblings, day care attendance, smoke exposure, parental atopic constitution, birth weight and educational level of the parents).

DISCUSSION

We found that in late preterm infants without atopic predisposition, severe RSV infection in early life is an independent risk factor for current wheeze at the age of six. To our knowledge, this is the largest published birth cohort reporting about the role of atopic predisposition in the link between severe RSV infection and current wheeze at school age.

It is known that atopy increases the risk of developing asthma. In addition, atopy is associated with an increased risk of RSV hospitalization in early life [19]. However, the precise interplay between atopy, RSV infection and asthma development is still unknown. Multiple explanations exists for the interaction between viral infections and atopy and their link to asthmatic disease. One explanation is that atopic disease alters the immune response towards an impaired response to viral infection which makes individuals more prone to (severe) infection [20]. Additionally, viral infections as well as atopic disease can cause bronchial epithelial damage which makes the lungs more susceptible to bronchial hyperreactivity [20]. Both the epithelial damage and altered immune response are key features in development of asthmatic disease. Our results are in line with the population-based study of Henderson et al. who showed that RSVH was only a risk factor in children without atopy [12]. However, RSVH did not increase the risk in children without atopy in other cohort studies by Sigurs and Zomer-Kooijker [9, 11]. Inconsistency with our results can potentially be explained by the limited sample size and different outcome definitions used in these studies. The MAKI trial, a randomized controlled trial in a similar patient population of late preterm infants, investigated whether RSV prophylaxis during infancy alters the risk of school age asthma. They found less parent-reported asthma at age six in children who received RSV prophylaxis but did not detect a difference in physician-diagnosed asthma or lung function [21]. Subgroup analysis for parental atopic disease

showed that RSV prophylaxis significantly reduced the risk of parent-reported current asthma in children without atopic parents, which is in line with our study. This reduced risk was also shown in an European/Canadian cohort study where RSV prophylaxis in children without atopic predisposition (absence of a family history of parental atopic disease), decreased the relative risk of recurrent wheezing by 80% [22]. Similar to our study, this reduction was not observed in children with an atopic background. In contrast, in a study in Japanese children, RSV-prophylaxis decreased recurrent wheeze only in children with an atopic background [8]. The discrepancy with the European/Canadian study was suggested to be caused by a fundamental difference between study populations in genetic makeup or the environment. Based on our study, we speculate that regardless the presence or absence of severe RSV infection, the atopic background on itself is sufficient to predispose children for the development of asthma. However, in the absence of an atopic predisposition, severe RSV infection seems to play a more important role in causing ongoing respiratory morbidity.

The strength of the current study is the prospective birth cohort design and large sample size, which allowed us to differentiate between children with and without atopic predisposition. There are also limitations worth mentioning. First, because we only included late preterm infants in our study, we cannot generalize our results to all infants. Wheezing disorders are known to be more prevalent in preterm infants compared to term born infants, and pathophysiologic mechanisms are likely to be different [23]. Second, bias could have been introduced during follow-up. Confounding bias is a problem in observational studies. We performed an extended literature review to select potential confounders for which we adjusted in analyses. Self-selection bias could have caused parents from children with current wheeze to respond more often because the study purpose was explained in the invitation email at six year follow-up. This could have resulted in a higher proportion of children with the outcome. In addition, to increase power we enriched the cohort with complete follow-up with RSVH cases from the non-response group resulting in more participants with the determinant. Additionally, attrition bias due to selective response in follow-up could have occurred since only 62% the participants with a valid email address completed follow-up. Participants with outdated contact information together with the non-responders comprised 55% of the total eligible study population. Surveys with long-term follow-up showed response rates varying from 41-63% [24-26]. Higher response rates were obtained when updated contact details were available, which was not the case in our study resulting in the inability to contact 651

eligible participants. Differences seen in the baseline characteristics between responders and non-responders indicated this attrition bias. We have corrected for all these biases that were introduced during follow-up by including covariates of 'missingness' and potential confounders in the regression model. Subsequently we have performed a sensitivity analysis using imputation to correct for missing outcome data in line with methodological literature [27]. All analyses showed comparable results which strengthens our believe that the conclusions drawn are valid. Third, because stratification in analyses was applied for both RSVH as well as atopic predisposition, numbers tend to become smaller. However, we observed consistent results in the complete case analysis and sensitivity analysis of all 2210 participants including 121 RSVH cases. This makes us feel confident that our conclusions are valid. Fourth, parents are known to overestimate wheezing in their child compared to when wheeze is assessed by a physician [28]. We chose parent-reported current wheeze over a doctor's diagnosis of asthma because our national guidelines dictate that no diagnosis of asthma can be ascertained in children below the age of six due to their inability to correctly perform spirometry. Moreover, no consent was given to collect medical data for children in the initial RISK study. It is therefore possible that the prevalence of parent-reported wheeze is overestimated in our study. However, if we compare average rates of wheeze at age six in literature, which range from 9.7 to 21.2% [8, 11, 12, 29], our average incidence of parent reported wheeze in the past 12 months (11.9%) is comparable. Moreover, our average incidence of the composite outcome current wheeze of 18.6% is very comparable to the average 19.0% described in the MAKI trial which used the same outcome definition [21]. Fifth, we did not collect data about how frequent the children in our study used their respiratory medication and experienced wheezing. The reported outcomes could therefore reflect a broad range of symptoms in the past 12 months. Last, we defined atopy of the child based on a parental history of atopic disease. We have chosen this definition since parental atopy is known to correlate with atopic disease in the offspring [30], and because it is readily available at birth and is therefore a useable prognostic factor in early life when the risk of severe RSV infection is highest.

CONCLUSION

In this prospective birth cohort study in healthy late-preterm infants we found that in children without atopic predisposition severe RSV bronchiolitis in the first year of life is an independent risk factor for current wheeze at age six. This suggests that RSV is

probably of less importance in the development of wheeze for children with an atopic predisposition since these children are already at increased risk. If reduction of ongoing respiratory symptoms in childhood can be realized by preventing severe RSV infection in certain risk groups, this could be a very viable solution to diminish the burden both in infancy as well as in later childhood. Especially in the light of new RSV vaccines currently being developed, future RSV prevention trials aiming to prevent ongoing respiratory symptoms should be analyzed separately for children with and without an atopic predisposition.

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SUPPLEMENTAL FILE:

RSV hospitalization in infancy increases the risk of current wheeze at age six in late preterm born children without atopic predisposition

Supplemental table S1. Risk factors for asthma and RSV hospitalization.

Variable:	Asthma risk factor*	RSV-hospitalization risk factor**	Confounder
Genetic/familial risk factors			
Atopic constitution in a first degree relative	Yes [1-10]	Yes [11-14]	**Yes**
Asthma in a first degree relative	Yes [15-20]	No	No
Inhalation medication used by parents	Yes [21]	No	No
Low SES/education level	Yes [4, 20-22]	Yes [23]	**Yes**
Single parent household	Yes [4]	No	No
Maternal age below 25 years	No	Yes [24]	No
Child specific risk factors			
Gender male	Yes [1-4, 6, 8, 18, 19, 21]	Yes [25]	**Yes**
No breastfeeding	Yes [2, 4, 6]	Yes [12, 26, 27]	**Yes**
Day care attendance	Yes [4, 20]	Yes [12]	**Yes**
Low birth weight	Yes [4]	Yes [24, 25]	**Yes**
Atopic constitution (eczema/rhinitis/ allergy)	Yes [3-8, 15-22, 28-30]	Yes [13]	**Yes**
Young age	No	Yes [31-34]	No
Prematurity	No	Yes [31, 32, 35]	No
Postterm delivery (>42 wGA)	Yes [21]	No	No
Chronic Lung disease (CLD)	No	Yes [36]	No
Broncho pulmonary dysplasia (BPD)	No	Yes [36]	No
Down syndrome	No	Yes [37]	No
Congenital heart disease	No	Yes [38]	No
Delivery by cesarean section	No	Yes [39]	No
Lower respiratory tract infection (LRTI)	Yes [1, 17, 21, 30, 40-42]	No	No
Wheezing (all types, situations and triggers)	Yes [3, 4, 7, 9, 10, 16, 19-21, 28, 40, 41]	No	No
Birth in proximity to RSV season	No	Yes [12, 24, 31, 43]	No
Vitamin D deficiency	No	Yes [44]	No
Environmental risk factors			
Maternal smoking during pregnancy	Yes [1, 4, 8]	Yes [25]	**Yes**
Parental smoking	Yes [8, 18]	Yes [11, 27]	**Yes**
Presence of siblings	Yes [4]	Yes [12, 25, 27, 45]	**Yes**
Urban environment	Yes [4, 22]	Yes [24]	**Yes**
Pets at home	Yes [20]	No	No

Supplemental table S1. Continued

Variable:	Asthma risk factor*	RSV-hospitalization risk factor**	Confounder
High altitude above 2500 m	No	Yes [46]	No
Climatic factors and air pollution	No	Yes [47-49]	No
Painting wall during pregnancy	Yes [22]	No	No
Fungus on wall at home	Yes [22]	No	No

[a] Based on a MEDLINE search for asthma prediction models using the search query: Asthma AND prediction AND (infant OR baby OR birth). In total 25 articles were included from which 11 articles were included from this initial search and 14 additional articles were found by checking references. The search was performed at the 7th of March 2017. [b] Based on table 4 in the review by Bont et al [50].

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CHAPTER 4

Defining asthma in children: how well do parents, doctors and spirometry agree?

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ABSTRACT

Rationale

Because diagnosing asthma in school-aged children is challenging, a variety of proxies for asthma are used in clinical practice and research settings as indicators of this disease.

Objectives

we aimed to provide insight in the agreement between various asthma indicators based on parental report, medical diagnosis, and spirometry.

Methods

Children from the WHISTLER birth cohort performed spirometry and were followed up with parental ISAAC questionnaires about asthma at age five and eight. Medical data was extracted from primary care records. We compared 15 asthma indicators based on parental report, medical diagnosis, and spirometry using positive agreement, Kappa statistics and latent class cluster analysis.

Results

At age five, 1007 children completed a study visit while 803 children visited at age eight. Depending on the indicator, the responder, and child's age, the asthma prevalence ranged from 0.2% to 26.6%. Cluster analysis revealed classes related to the presence of recent symptoms, and a decreased lung function. Agreement between parents and doctors was generally low with Kappa coefficients ranging from 0.07 (recent wheeze) to 0.52 (recent asthma medication). Additionally, parental report showed to be sensitive to recall bias over time.

Conclusions

Dependent on the asthma indicator, the responder, and the age of the child, substantial differences in agreement were observed between commonly used indicators associated with asthmatic disease in school-aged children. Most agreement between parents and doctors was seen for objective and recent indicators such as the recent use of asthma medication. We advocate caution when literature with different asthma indicators is compared.

INTRODUCTION

Worldwide, over 358 million people are estimated to have asthma making this disease one of the most common chronic diseases [1]. The prevalence of asthma based on national registries ranges from 1.7 to 13.5% in western countries [1-3]. While some variation can be explained by differences in region and study population, the use of proxies for medical diagnoses also likely contributes to differences in asthma prevalence [4]. There are a variety of proxies for asthma, as shown in a systematic review of 122 published articles which found 60 different indicators [4]. Moreover, it is increasingly acknowledged that asthma is an umbrella term that includes several phenotypes. Differences between indicators of asthma can therefore also reflect different phenotypes of this disease. While it is still largely unknown whether these indicators reflect a similar condition and whether it is justifiable to compare results from studies using different indicators, we hypothesize that much of the variability in the prevalence of asthma is a result of how it is defined and measured. Knowing which asthma indicators are related and which ones differ is important to facilitate a better comparison of published literature as well as aid to the selection of appropriate endpoints for defining asthma in future studies. Moreover, knowledge about the agreement between different indicators is not only useful for research, but is also required to ask the right questions to parents in order to obtain the most meaningful information in clinical practice. In this study we aim to investigate the agreement between indicators that are commonly used in literature to define asthmatic disease based on parental report to determine how they relate to indicators based on medical diagnosis and lung function in children aged five and eight years old.

METHODS

Study population

This study was performed as part of the WHeezing and Illnesses STudy LEidsche Rijn (WHISTLER), an ongoing prospective birth-cohort study on determinants of wheezing illnesses. Healthy term born infants born between December 2001 and December 2012 living in the Leidsche Rijn district of Utrecht the Netherlands were enrolled within two months after birth. Exclusion criteria at baseline were gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease. The study design and rationale of WHISTLER are described in detail elsewhere [5]. The WHISTLER project was approved by the medical ethical committee of the University Medical Center Utrecht, the Netherlands. Written informed consent was obtained from the parents.

Data collection

At the age of five all children that were initially enrolled in the birth cohort were invited for a study visit during which lung function was assessed using spirometry. A maximum of 1000 participants were recruited for follow-up at age five. Spirometry was performed using a calibrated spirometer (ZAN 100 spirometer, nSpire USA) and all children withheld their rescue medication for at least 12 hours beforehand [5] If the child had suffered from a respiratory tract infection in the last 2 weeks, the test was postponed. Maximal flow-volume curves were measured according to the ATS/ERS standards [6]. The largest forced expiratory volume in 0.5 second (FEV0.5), 1 second (FEV1) and forced vital capacity (FVC) were selected from three correctly performed assessments. Additionally, standardized ISAAC questionnaires about atopic diseases and asthmatic symptoms were filled out by the parents during these study visits irrespective of a successful lung function test. Medical data about doctor's visits and the use of medication by these children was extracted from the general practitioners' electronic medical database using the International Classification of Primary Care (ICPC) [7] and the Anatomical Therapeutical Chemical (ATC J01) coding systems. Children that completed five year follow-up were subsequently invited for a similar second study visit at the age of eight.

Indicators of asthma

Commonly used proxies of asthmatic disease were selected for this study based on literature and availability in WHISTLER [4]. In this paper, we will refer to these proxies as "indicators of asthma" because of their presumed association with the risk of asthmatic disease. Clinical indicators based on either parental opinion or medical diagnosis included;

current asthma, ever asthma, current wheeze, ever wheeze, and the current use of asthma medication. The time constraint for 'current' was within the past 12 months while 'ever' was defined as a history of asthma/wheeze at some moment in their life. Parentally reported indicators were based on the ISAAC questionnaire while their equivalents from medical diagnosis were based on ICPC and ATC codes. Indicators of a lung function deficit (FEV0.5, FEV1, FVC, FEV0.5/FVC and FEV1/FVC) were dichotomized at the lower limit of normal (LLN; below the 5th percentile of predicted) using the Dutch normative values which are corrected for age, length and gender [8]. The exact definitions of all indicators used in this article can be found in the online data supplement [Table S1].

Statistical analysis

First, we analysed the cross-sectional agreement between different indicators at age five and eight. We determined agreement between indicators in three ways to provide a balanced view about their relations. Positive agreement was calculated to provide unilateral agreement between two variables. Cohen's kappa coefficients were calculated to determine bilateral agreement. Additionally, we performed latent class cluster analysis at age five to study the agreement between multiple indicators by estimating clusters or "classes" of indicators of asthma. While latent class analysis can be used for the purpose of defining phenotypes of disease, our aim of the analysis was specifically to determine agreement between de included indicators of asthma. Proportional class membership probabilities of the included indicators in the final latent class cluster model were obtained to measure this agreement. Indicators within the same latent class are homogeneous, while indicators in different latent classes are dissimilar from each other. Clustering of indicators was graphically displayed in a Tri-plot and bar chart. Detailed information about latent class cluster analysis, model development and model selection can be found in the supplemental file [Supplement Latent class cluster analysis].

Second, we analysed intra-subject longitudinal consistency between age five and eight. Longitudinal agreement was analysed by estimating Cohen's kappa coefficients and persistency and development of new cases for the same indicator between age five and eight. No imputation of missing data was performed. Latent class analysis was performed using Latent Gold version 5.1 (Statistical Innovations Inc.) while other analyses were performed in R version 3.5.1.

RESULTS

Participants

Of the 2443 children who initially participated in WHISTLER, 1144 children at the age of five, and 1127 at the age of eight had data available from at least one source (study visit and/or registry data) [Figure 1]. At age five, 1007 children were invited for a study visit of whom 992 completed the questionnaire and 940 attended the clinic for spirometry. Of those that visited the clinic 39 refused spirometry, 32 were technically unable to correctly execute the procedures, and 72 results were not reproducible resulting in 792 correctly performed assessments. At age eight, 803 children were invited for a study visit of whom 782 completed the questionnaire and 719 attended the clinic. Of those that visited the clinic 1 refused, 9 assessments were technically insufficient and 28 were not reproducible, resulting in 681 correctly performed assessments. Baseline characteristics of participants in various stages of follow-up are shown in Table 1 and data availability is shown in Figure 1.

Table 1. Baseline characteristics

	Total cohort (N=2443)	At 5 year follow-up (N=1007)	At 8 year follow-up (N=803)
Gender male	1191/2443 (49%)	485/1007 (48%)	370/803 (46%)
Age in months (median, ±SD)	-	65 (+/- 4.6)	107 (+/- 11.6)
Birth weight in grams (mean, ±SD)	3544 (+/- 500g)	3552 (+/- 498g)	3561 (+/- 481g)
wGA (mean wks+days, ±SD)	39+6 (+/- 9 days)	39+6 (+/- 9 days)	39+6 (+/- 9 days)
Breastfed [a]	1320/2439 (54%)	521/915 (57%)	430/742 (42%)
Siblings	1134/2443 (46%)	421/916 (46%)	353/743 (48%)
Day care attendance [a]	2367/2440 (97%)	893/915 (98%)	723/742 (97%)
Pre-natal smoke exposure [b]	318/2427 (13%)	160/912 (18%)	118/740 (16%)
Smoking parents (at least one)	415/2008 (21%)	173/756 (23%)	141/632 (22%)
Asthmatic mother	184/2125 (9%)	74/808 (9%)	62/675 (9%)
Asthmatic father	116/2019 (6%)	48/769 (6%)	37/644 (6%)
Allergic mother [c]	802/2152 (37%)	310/815 (38%)	257/681 (38%)
Allergic father [c]	785/2040 (39%)	310/774 (40%)	262/650 (40%)
High educational level mother [d]	1473/2105 (70%)	551/803 (69%)	453/671 (68%)
High educational level father [d]	1284/2002 (64%)	452/766 (59%)	381/640 (60%)

Abbreviations: wGA = weeks gestational age. [a] At least one month in the first year of life. [b] either maternal smoking or exposure of the mother to smoke. [c] Reported hay-fever or allergy for house-dust mite, animals or food. [d] Educational level higher than secondary school.

Prevalence

The prevalence of the clinical indicators of asthmatic disease at the ages of five and eight as reported by parents or based on medical diagnosis ranged from 0.2% (medically-diagnosed current wheeze at age eight) to 26.6% (parent reported ever wheeze at age five) [Table 2]. The incidence of medically-diagnosed recent wheeze declined rapidly after the first years of life based on the ICPC database with incidences of 4.4%, 2.2%, 1.2% and 1.2% in the first four years of life respectively. Based on spirometry, the prevalence of a lung function deficit below the lower limit of normal ranged from 4.4% to 7.6% [Table 3].

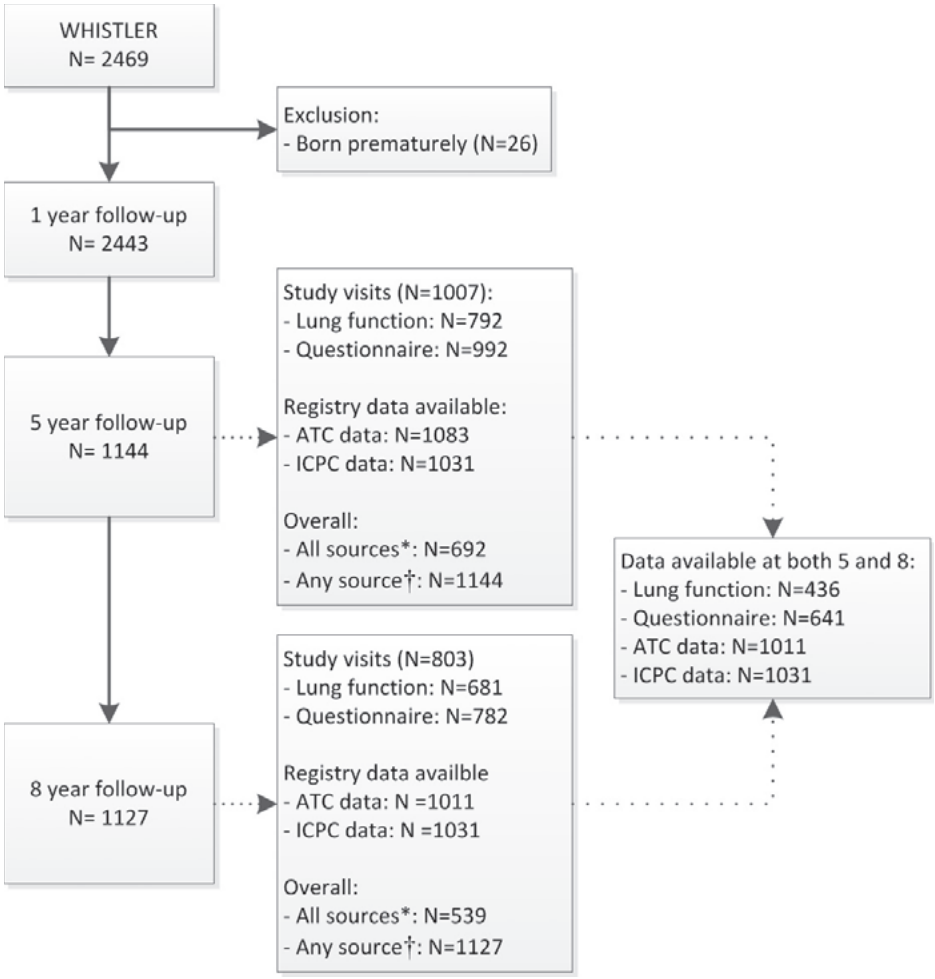

Figure 1. Flowchart
* Data available from all data sources (questionnaires, lung function assessment, ATC and ICPC registries). † Data available from at least 1 data source.

Table 2. Prevalence of indicators at age five and eight

	Age five		Age eight	
	Parental-reported	Medical diagnosis	Parental-reported	Medical diagnosis
Indicator [a]	N/total (%)	N/total (%)	N/total (%)	N/total (%)
Current asthma	53/917 (5.8%)	16/1031 (1.6%)	12/282[†] (4.3%)	19/962 (2.0%)
Asthma (ever)	68/974 (7.0%)	75/1031 (7.3%)	71/772 (9.2%)	98/1031 (9.4%)
Current wheeze	73/976 (7.5%)	4/1031 (0.4%)	43/776 (5.5%)	2/962 (0.2%)
Wheeze (ever)	260/978 (26.6%)	50/1031 (4.8%)	154/777 (19.8%)	55/1031 (4.8%)
Current asthma medication	37/918 (4.0%)	68/1083 (6.3%)	9/286 [b] (3.1%)	28/1011 (2.6%)

Denominators may change due to differences in missing values, based on the source of report and age of the child. [a] see Table E1 for the exact definitions. [b] Questions about current asthma and current asthma medication were rephrased during follow-up resulting in only a minority of participants at age eight who answered to the same question as compared to age five.

Table 3. Prevalence of lung function deficit at age five and eight

	Age five	Age eight
Indicator [a]	N/total (%)	N/total (%)
FEV0.5(<LLN)	47/777 (6.0%)	44/581 (7.6%)
FEV1 (<LLN)	53/792 (6.7%)	46/676 (6.8%)
FVC (<LLN)	57/792 (7.2%)	51/676 (7.5%)
FEV0.5/FVC (<LLN)	47/776 (6.1%)	35/581 (6.0%)
FEV1/FVC (<LLN)	49/791 (6.2%)	30/675 (4.4%)

Abbreviations: LLN = lower limit of normal or below the 5th percentile of predicted. FEV = Forced Expiratory Volume in either half (FEV0.5) or 1 second (FEV1), FVC = Forced Vital Capacity. [a] see Table E1 for the exact definitions.

Agreement at age five and eight

Agreement between parents and doctors at age five is displayed in Figure 2-3. Unilateral positive agreement showed a very poor agreement for recent wheeze (4% agreement) but a good agreement of 75% for the recent use of asthma medication [Figure 2]. This was confirmed by kappa's ranging from 0.07 (no agreement) for recent wheeze to 0.52 (moderate agreement) for asthma medication [Figure 3]. Most agreement between parents and doctors was seen for recent asthma and recent asthma medication use. The associations observed at age five between recent asthma and recent asthma medication were confirmed at eight years of age [Supplemental figure S1 and S2].

Generally poor agreement was seen between lung function indicators and the clinical indicators as reported by parents at age five [Figures 2-3]. Although agreement was

slightly better at age eight, the kappa statistics indicated "fair" agreement at best [Supplemental figure S1-2]. Despite this low agreement, children with a clinical label of asthma did show a decreased lung function compared to those without an asthmatic label [Online supplement Table S2]. However, most children still remained within the limits of normality.

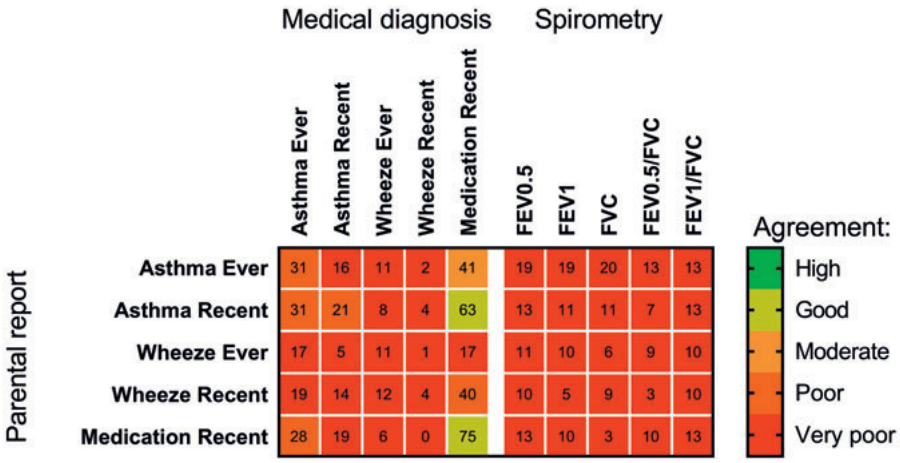

Figure 2. Agreement at age five (positive agreement).
Positive agreement is shown unilaterally for the horizontal indicator compared to the vertical indicator. For example; a parental report of recent asthma medication is in 75% of the cases confirmed in the doctor's registry. Lung function deficit is dichotomized based on the lower limits of normal (<5th percentile).

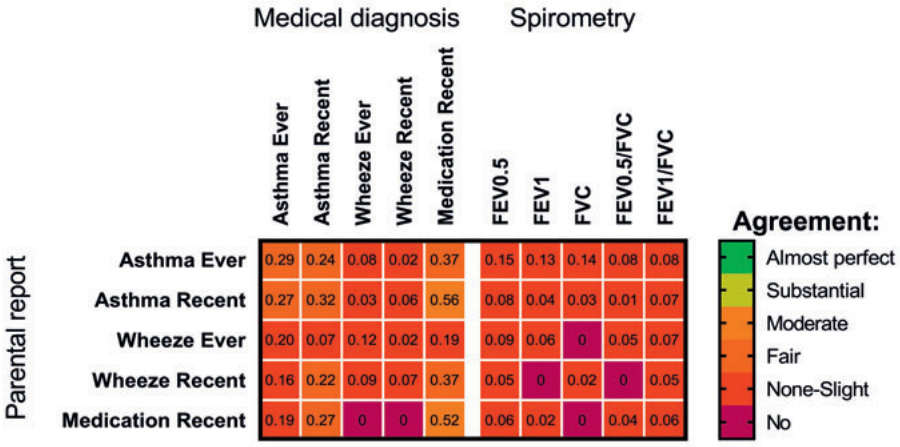

Figure 3. Agreement at age five (Kappa).
Cohen's kappa's show bilateral agreement between the indicators. For example the agreement between recent medication use for parents and doctors is 0.52 indicating moderate agreement. Lung function deficit is dichotomized based on the lower limits of normal (<5th percentile).

Cluster analysis at age five

A four-class model best fitted the underlying agreement in the data in the latent class cluster analysis. Details about the model development, selection process and the individual class membership probabilities can be found in the supplemental file [Supplement Latent class cluster analysis, Figure S3]. Clustering of indicators based on these probabilities was graphically displayed in the Tri-plot shown in Figure 4. Negative answers to all of the included indicators (e.g. not having recent asthma) clustered together and were collectively labelled as cluster "No asthma" which comprised 80% of the participants. The second cluster consisted of indicators of recent asthmatic symptoms and included 9% of the participants. Cluster three included indicators based on a lung function deficit and included 6% of the participants. Lastly, some indicators did not have a clear predominance to be assigned to either of the clusters and remained in the middle of the plot. These included indicators of a history of symptoms and a diminished FEV0.5.

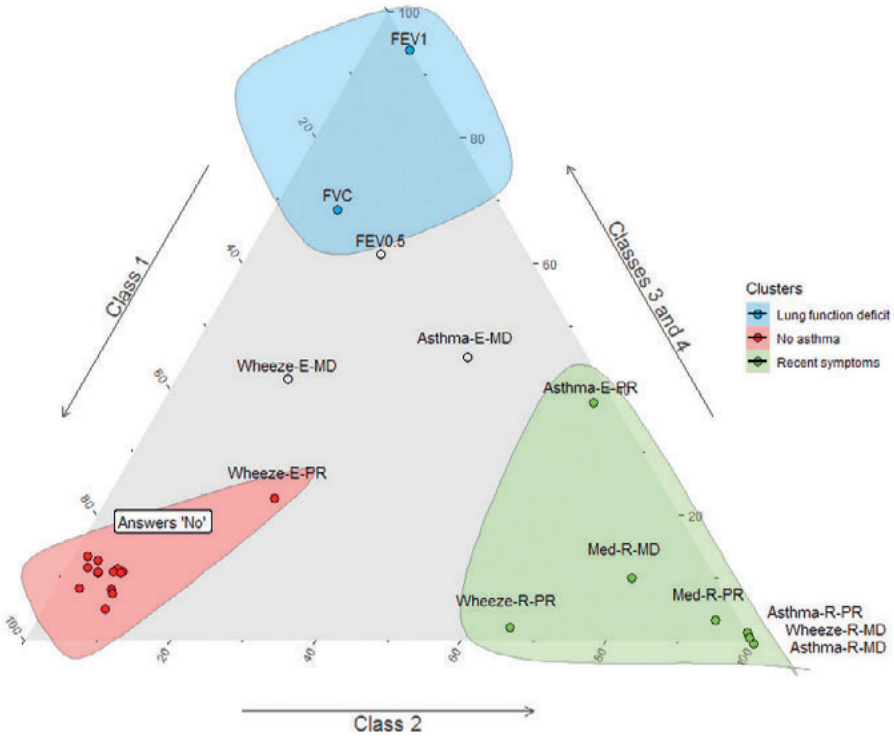

Figure 4. Tri-plot of class membership probabilities and clustering of indicators at age five. Abbreviations: PR = Parent-Reported, MD= Medical diagnosis, E = Ever, R = Recent. The Tri-plot indicates the class membership probabilities and clustering by arranging all proportional class membership probabilities of a variable as one data point in a triangular plane in which each point of the triangle represents a class. Probabilities for classes 3 and 4 are summed together in the top of the triangle to form the Tri-plot. Variables are coloured and encircled based on their highest probability for one of the four classes. If variables did not have a clear predominance (<50% probability to be assigned to either one of the classes), they were not coloured. Cluster names are based on the majority of indicators in that circle.

Longitudinal agreement

Based on parental report, indicators of a history of symptoms were most constant over time (54-58% consistency) [Table 4]. However, nearly half of these "ever" diagnoses reported at age five were therefore not reported anymore at age eight, indicating a substantial loss in the recollection of these past symptoms over time by parents. The indicator that showed most consistency for ongoing current symptoms was the presence of current asthma both based on parental opinion (41,2% consistency) as well as based on medical diagnosis (25.0% consistency). Lung function was relatively constant over time as indicated by 20% to 45% consistency of these indicators.

Table 4. Longitudinal consistency between the age of five and eight years old.

Indicator [a]	Prevalence age five N/total (%)	Prevalence age eight N/total (%)	Persistent cases [b] N/total (%)	New cases [c] N/total (%)	Cohen's kappa
Parent-reported:					
Current wheeze	47/625 (7.5%)	29/625 (4.6%)	12/47 (25.5%)	17/29 (58.6%)	0.27
"Ever" wheeze	168/626 (26.8%)	118/626 (18.8%)	91/168 (54.2%)	27/118 (22.3%)	0.53
Current asthma [d]	17/273 (6.2%)	10/273 (3.7%)	7/17 (41.2%)	3/10 (30.0%)	0.50
"Ever" asthma	41/621 (6.6%)	50/621 (8.1%)	29/41 (58.0%)	21/50 (42.0%)	0.61
Current asthma medication [d]	12/276 (4.3%)	8/276 (2.9%)	3/12 (25.0%)	5/8 (62.5%)	0.27
Medical diagnosis:					
Current wheeze	4/962 (0.4%)	2/962 (0.2%)	0/4 (0%)	2/2 (100%)	0
"Ever" wheeze	50/1031 (4.8%)	55/1031 (5.3%)	50/50 (100%)	5/55 (9.1%)	0.95
Current asthma	16/962 (1.7%)	19/962 (2.0%)	4/16 (25.0%)	15/19 (78.9%)	0.21
"Ever" asthma	75/1031 (7.3%)	98/1031 (9.5%)	75/75 (100%)	23/98 (23.5%)	0.86
Current asthma medication	68/1011 (6.7%)	28/1011 (2.8%)	13/68 (19.1%)	15/28 (53.6%)	0.24
Lung function (<LLN):					
FEV0.5	20/357 (5.6%)	29/357 (8.1%)	9/20 (45.0%)	20/29 (69.0%)	0.32
FEV1	28/432 (6.5%)	32/432 (7.4%)	12/28 (42.9%)	20/32 (62.5%)	0.36
FVC	30/432 (6.9%)	30/432 (6.9%)	8/30 (26.7%)	22/30 (73.3%)	0.21
FEV0.5/FVC	20/357 (5.6%)	22/357 (6.2%)	4/20 (20.0%)	18/22 (81.8%)	0.14
FEV1/FVC	25/431 (5.8%)	22/431 (5.1%)	8/25 (32.0%)	14/22 (63.6%)	0.30

Abbreviations: LLN = lower limit of normal or below the 5th percentile of predicted. FEV = Forced Expiratory Volume in either half (FEV0.5) or 1 second (FEV1), FVC = Forced Vital Capacity. Denominators may have changed compared to other tables because only cases were included that had data available at both time points. [a] see Table E1 for the exact definitions. [b] Cases that were identified at age five that still reported symptoms at age eight. [c] New cases compared to those identified at age five. [d] Questions about current asthma and current asthma medication were rephrased during follow-up resulting in only a minority of participants at age eight who answered to the same question as compared to age five.

DISCUSSION

This study aimed to provide insight into the agreement of the wide variety of indicators of asthmatic disease used in clinical practice and research settings to define asthma in children. Considerable variance in agreement between responders as well as large variance in the prevalence was observed when common indicators of childhood asthma were compared. Our analysis revealed distinct clusters of more comparable indicators, namely those that indicated the presence of current symptoms and indicators of a lung function deficit. Additionally we observed substantial problems in the recollection by parents of a history of symptoms.

Disagreement between parents and doctors in reporting asthmatic disease has been observed before [9-14]. Parents report higher rates of a history of wheeze and asthma compared to doctors [9, 10, 14]. We observed that the incidence of medically-attended wheeze decreases significantly after the first years of life and is nearly absent at school-age. However, these symptoms still persist based on parental report. Based on parental report, the yearly number of episodes of wheeze in children with persistent wheeze was comparable at age five and eight. We therefore hypothesize that the inconsistency between parental and doctor report could be the result of parents getting familiar with their child's symptoms and do no longer seek medical attention. Parental report of a history of (doctor's diagnosed) asthma has been suggested as a stable indicator of childhood asthma over time and reflected most agreement with the prevalence obtained by medical diagnosis [13, 15, 16]. Although we also confirm this agreement, we do show that there is significant recall bias based on parental report for a history of asthmatic disease. This recall bias combined with the poor agreement we observed with the presence of current symptoms can in our opinion strongly diminish the clinical value of using these life-time indicators. Moreover, these "ever" indicators showed to have less probability to fall in one of the classes in our analysis suggesting they are more representative of children in which symptoms are in remission or resolved. Similar to our findings, a higher agreement between parents and doctors was seen for recent objective indicators such as the recent use of asthma medication [11, 12]. Positive agreement for recent use of respiratory medication in these studies ranged from 64% [11] to 87% [12] which is comparable to the 75%-78% at age five and eight we observed. We observed poor agreement between lung function and clinical symptoms. Although counterintuitive, this mismatch between a clinical diagnosis of asthma and lung function tests has been

reported before [17], as well as disagreement between current symptoms and lung function as indicators of asthma severity [18, 19]. Although we observed a decreased lung function in children with an asthmatic label, the majority of asthmatic children still had a lung function within the boundaries of normality. Cut-off values such as the LLN are perhaps too strict in children which also matches with the observation that the commonly used cut-off values for FEV1 and the FEV1/FVC ratio do not match well with clinical asthma in children [17].

Strengths of our study are the cohort size and the availability of different sources of asthma-related data at both the ages of five and eight. This allowed for comparing a variety of commonly used indicators based on parent-report, medical diagnosis and spirometry. Repeated measurements at age five and eight allowed us to determine the temporal relations of various indicators during this crucial period of development. We used multiple statistical methods to analyse agreement which showed uniform results.

Limitations have to be discussed as well. First, although coverage of primary care visits in this database is very complete, the use the registration codes for obtaining a medical diagnosis could have attributed to a lower incidence of medical diagnoses compared to what was reported by parents because registries can be less sensitive for mild symptoms [20]. One could imagine that an episode of wheeze, if triggered by a respiratory infection, is coded as respiratory infection rather than wheeze. Free text analysis of the clinical records would have been more sensitive but was unfortunately unavailable in this study. Second, the WHISTLER cohort was overrepresented with families with a high socioeconomic status (SES) compared to the district average. Although evidence is conflicting, more studies point towards an increased burden of asthma in low-SES children [21]. Asthma incidence could therefore be underestimated in our study. Since primary care is freely accessible in the Netherlands, we do not expect that SES disproportionally affected the medically-diagnosed indicators compared to the parental-reported indicators. Therefore, we believe that it is unlikely that SES have affected the underlying agreement between the indicators when assumed that all are evenly underestimated. Third, only approximately 40% of the initial study population of WHISTLER participated in follow-up at age five and 33% completed follow-up at the age of eight. Although planned, this selective follow-up could introduce selection bias. However, since baseline characteristics of participants in various stages of follow-up did not show major differences we believe that the risk of having selected a different subpopulation of our cohort for follow-up is small. Fourth,

not all sources of report were available for all participants. Data was not so much missing but rather 'absent' in the presence of more data from an alternative source such as a medical record. We were therefore unable to compare all data from different sources of information for all individual participants. We chose not to impute missing data if no direct comparison could be made since this would imply that we had to impute complete study visits when only medical registry data was available. Also, with less data available at age eight, the results of the analyses at the age of five are more robust. Fifth, we did not to evaluate reversibility or performed challenge tests during spirometry in our cohort of otherwise healthy children which may have attributed to the low agreement we observed compared to the clinical endpoints. However, this disagreement has also been reported in a study that did compare between reported symptoms and results from various lung function tests [17]. Last, we describe agreement and differences between indicators of asthmatic disease but did not investigate the clinical implications of having such a label. For clinical relevance, it would be interesting to know which of the indicators are more correlated with a diminished quality of life or require more medical care. This knowledge could aid in the selection of endpoints that are not only agreed upon, but are also clinically relevant.

CONCLUSION

Dependent on the indicator, the responder, and the age of the child, we found substantial differences in agreement and prevalence between various commonly used indicators associated with asthmatic disease in school-aged children from an unselected birth cohort. These data advocate caution when literature with different asthma indicators is compared. Based on these results, we advise the use recent and objective indicators, such as a recent asthma diagnosis and the recent use of asthma medication because they displayed the highest agreement between parents and doctors with the lowest risk of recall bias over time.

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SUPPLEMENTAL FILE:

Defining asthma in children: how well do parents, doctors and spirometry agree?

Supplemental Table S1. Definitions of asthma indicators used for analyses

Parental-reported	Medical diagnosis	Spirometry [a]
Current asthma (in past 12 months): "Has your child suffered of the following condition (diagnosed by a physician) in the past 12 months: asthma / bronchitis"	**Current asthma (in past 12 months):** Consultation with asthma (in past 12 months), Asthma = ICPC code R96 (R96; asthma, R96.01; hyperreactive airways, R96.02; allergic asthma)	-
Asthma (ever): "Has your child ever had asthma?"	**Asthma (ever):** Consultation with asthma (ever): Asthma = ICPC code R96 (R96; asthma, R96.01; hyperreactive airways, R96.02; allergic asthma)	-
Current asthma medication: "Did your child use medication prescribed by a physician in the past 3 months?" All names of the used medicines were thereafter noted which was used to select respiratory inhalation medication. Current asthma medication was defined as the use of beta-mimetics and/or inhalation corticosteroids in the previous 3 months.	**Current asthma medication:** Active prescription of asthma medication (past 12 months [b]): Asthma medication based on ATC R03B/R03A codes (beta-mimetics = ATC R03A and/or inhalation corticosteroids = ATC R03B). Current asthma medication was defined as a prescription of beta-mimetics and/or inhalation corticosteroids in the past 12 months	-
Current wheeze (in the past 12 months): "Has your child ever had wheezing or whistling in the chest in the past 12 months?"	**Current wheeze (in the past 12 months):** Consultation for wheeze (in the past 12 months), wheeze = ICPC code R03 (R03; wheezing)	-
Wheeze (ever): "Has your child ever had wheezing or whistling in the chest at any time in the past?"	**Wheeze (ever):** Consultation with wheeze (ever), wheeze = ICPC code R03 (R03; wheezing)	-
-	-	Decreased FEV0.5
-	-	Decreased FEV1
-	-	Decreased FVC
-	-	Decreased FEV0.5/FVC
-	-	Decreased FEV1/FVC

Abbreviations: LLN = lower limit of normal or below the 5th percentile of predicted. FEV = Forced Expiratory Volume in either half (FEV0.5) or 1 second (FEV1), FVC = Forced Vital Capacity. **[a]** below the lower limits of normal (LLN or below the 5th percentile), obtained using the Dutch normative values which are corrected for age, length and gender [8] **[b]** Since most aerosols have an expiry date that can easily exceed 3 months, the wider time constraint of 12 months is used for medically prescribed asthma medication to compare to the usage of asthma medication in the last 3 months as reported by parents.

Supplemental Table S2. Lung function stratified by clinical asthma at age five.

Lung function	Never asthma (parent report)	Asthma "Ever" (parent report)	p-value
FEV0.5 (< LLN, N (%))	35/693 (5.1%)	10/54 (18.5%)	<0.001
FEV1 (< LLN, N (%))	43/708 (6.1%)	10/54 (18.5%)	0.001
FVC (< LLN, N (%))	45/708 (6.4%)	11/54 (20.4%)	<0.001
FEV0.5/FVC (< LLN, N (%))	37/692 (5.3%)	7/54 (13.0%)	0.02
FEV1/FVC (< LLN, N (%))	40/707 (5.7%)	7/54 (13.0%)	0.03
FEV0.5 percentage predicted mean (±SD)	101% (14.4%)	93% (15.3%)	<0.001
FEV1 percentage predicted mean (±SD)	100% (13.1%)	94% (13.5%)	0.001
FVC percentage predicted mean (±SD)	102% (15.5%)	97% (14.3%)	0.03
FEV0.5/FVC percentage predicted mean (±SD)	99.8% (11.4%)	96% (13.9%)	0.09
FEV1/FVC percentage predicted mean (±SD)	98% (6.0%)	96 (8.3%)	0.09
Lung function	No current asthma (parent report)	Current asthma (parent report)	p-value
FEV0.5 (< LLN, N (%))	40/704 (5.7%)	6/45 (13.3%)	0.04
FEV1 (< LLN, N (%))	48/710 (6.8%)	5/45 (11.1%)	0.27
FVC (< LLN, N (%))	52/710 (7.3%)	5/45 (11.1%)	0.35
FEV0.5/FVC (< LLN, N (%))	41/703 (5.8%)	3/45 (6.7%)	0.82
FEV1/FVC (< LLN, N (%))	41/709 (5.8%)	6/45 (13.3%)	0.04
FEV0.5 percentage predicted mean (±SD)	101% (14.7%)	96% (13.2%)	0.01
FEV1 percentage predicted mean (±SD)	100% (13.4%)	97% (12.2%)	0.11
FVC percentage predicted mean (±SD)	101% (15.6%)	100% (13.7%)	0.40
FEV0.5/FVC percentage predicted mean (±SD)	100% (11.7%)	97% (11.2%)	0.07
FEV1/FVC percentage predicted mean (±SD)	98% (6.1%)	97% (7.3%)	0.35

Abbreviations: LLN = lower limit of normal or below the 5th percentile of predicted. FEV = Forced Expiratory Volume in either half (FEV0.5) or 1 second (FEV1), FVC = Forced Vital Capacity.

Latent class cluster analysis; model building, selection process and probabilities

Background

While latent class cluster analysis can be used to define phenotypes or clusters, latent class analysis can also be used to measure agreement, and is increasingly done so in the field of diagnostic accuracy studies when there is no gold standard to estimate the sensitivity and specificity of index tests in relation to unmeasured, unknown "latent" disease status. In latent class cluster analysis it is assumed that the manifest (measured) variables are all individual classifiers for an underlying latent (unmeasured) disease status. The latent disease status is a categorical variable with a yet to be defined number of classes, that reflect those having or not having the disease, or particular subtypes of the disease. These classes are defined by the patterns observed in the data. Depending on the diversity of the data, a number of classes that best matches the underlying structure

of the data is modelled. This model provides probability-based classifications to each latent class which means that given a participants' set of responses, there is a certain probability to be classified in a certain latent class. These class membership probabilities provide information on which variables are strongly or weakly characteristic of certain classes. Cases within the same latent class are homogeneous on certain criteria (measured variables), while cases in different latent classes are dissimilar from each other.

Model selection methods

All ten clinical indicators (five parental, five medically diagnosed; Table S1) as well as FEV0.5, FEV1 and FVC were included in the latent class model. Since the lung function ratios (FEV0.5/FVC and FEV1/FVC) were prone to overlap with their individual markers (FEV0.5, FEV1, FVC) this could disturb the model. For similar reasons we chose not to include composites of clinical indicators of asthma. To select the best fitting latent class cluster model we evaluated the range of latent class cluster models with increasing numbers of latent classes using the Bayesian information criterion (BIC) and the Akaike information criterion 3 (AIC3) [22, 23]. Within the selected range of different class models we performed post-hoc correction for local dependencies for each class model. Local dependencies are correlations between indicators that could not be explained by the underlying classes and can therefore violate the main assumption of conditional independence, stating that variables are only correlated through their relation with the underlying latent class. By adding direct effects to the model for these local dependencies you can correct for this potential source of biased results. Local dependencies can be observed by checking the bivariate residuals and correcting for those that are increased. Lastly, after optimizing the different class models, the bootstrapped likelihood ratio test (BLRT) was used to define the optimal model by comparing two different class models with a significance level of p=0.05 [24].

Results

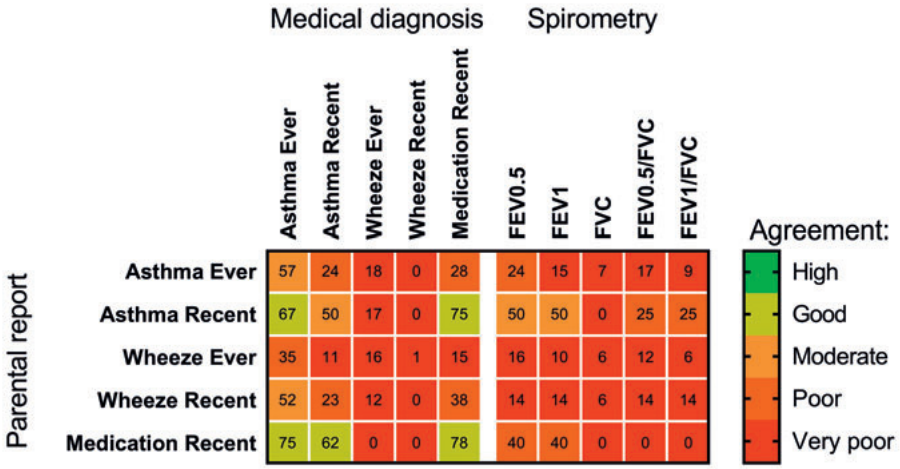
Model fit statistics for models between one and five latent classes were compared [Supplemental Table S3]. The information criteria suggest a range between a three-class model (BIC) and a four-class model (AIC3). The p-values of the BLRT after correction for local dependencies were significant up to and including a four-class model. Therefore, the four-class model was chosen, which was also in line with the clinical interpretability of the classes. The individual class membership probabilities for the four classes per asthma indicator are shown in Supplemental Figure S3.

Supplemental Table S3. Model fit statistics for latent classes

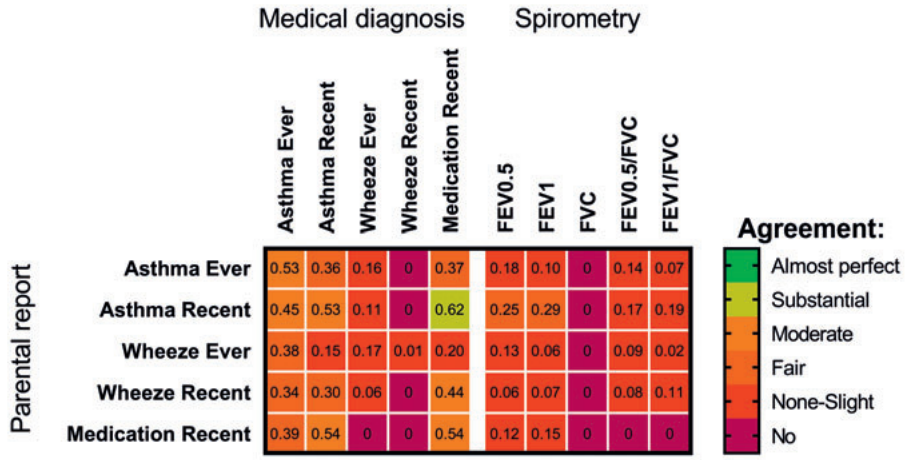
	BIC	AIC3	No. parameters	P-value BLRT
1-class	3988	3943	13	NA
2-class	3462	3369	27	NA
3-class	3356	3214	41	NA
4-class	3360	3170	55	NA
5-class	3408	3170	69	NA
After correction for local dependencies				
3-class corrected	3252	3097	45	NA
4-class corrected	3294	3093	58	0.000

Abbreviations: NA = Not applicable, BIC = Bayesian information criterion, AIC3 = Akaike information criterion 3, BLRT = bootstrap likelihood ratio test

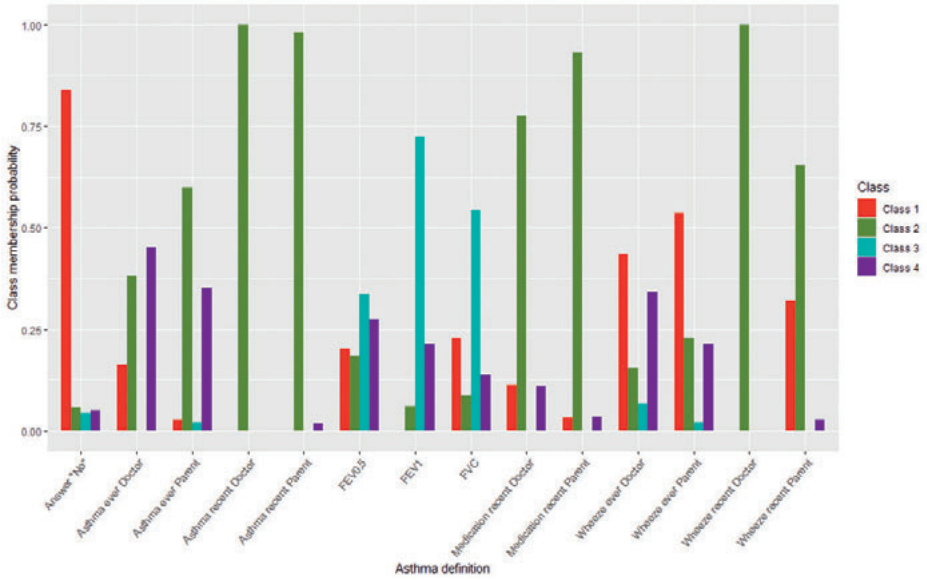
SUPPLEMENTAL FIGURES



Supplemental Figure S1: Agreement at age eight (positive agreement). Positive agreement is shown unilaterally for the horizontal indicator compared to the vertical indicator. For example; a parental report of recent asthma medication is in 78% of the cases confirmed in the doctor's registry. Lung function deficit is dichotomized based on the lower limits of normal (<5th percentile).



Supplemental Figure S2: Agreement at age eight (Kappa). Cohen's kappa's show bilateral agreement between the indicators. For example the agreement between recent asthma between parents and doctors is 0.53 indicating moderate agreement. Lung function deficit is dichotomized based on the lower limits of normal (<5th percentile).



Supplemental Figure S3. Class membership probabilities per indicator at age five. The bar chart indicates the individual probabilities of classification to a certain class per indicator included in the analysis. This figure is the basis of the tri-plot (Figure 4).

PART II
RSV in older adults

CHAPTER 5

Burden of respiratory syncytial virus infection in community-dwelling older adults in Europe (RESCEU): an international prospective cohort study

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ABSTRACT

Background

Respiratory syncytial virus (RSV) infection in older adults is recognized as an important health issue. We aimed to assess the community burden of RSV in Europe in older adults aged ≥60 years.

Methods

This international prospective observational cohort study is part of REspiratory Syncytial virus Consortium in EUrope (RESCEU). Participants were recruited before two independent RSV-seasons through general practitioner's offices. Participants reported weekly about symptoms of acute respiratory tract infection (ARTI) during one RSV-season. ARTI patients were tested for RSV during home visits and completed a daily symptom diary. RSV-illness included PCR-confirmed ARTI and those showing seroconversion over the season. RSV-ARTI was based on PCR alone (ClinicalTrials.gov, NCT03621930).

Results

We recruited 1040 participants (527 in season 2017-2018, 513 in season 2018-2019) with a median age of 75 years (range 60-100). 1023 (99%) lived independently at home at baseline. RSV-illness incidence was 4.2% (22/527) and 7.2% (37/513) in the respective seasons. RSV-illness did not affect frailty or cardiopulmonary status during the course of the study. No patients were hospitalized or died from RSV-illness. In the 36 patients with PCR confirmed RSV-ARTI, symptom duration averaged 19 days, while a doctor's visit took place in 11/36 (31%) of cases. RSV-ARTI could not clinically be differentiated from all other ARTI based on symptoms.

Conclusion

This European study showed that RSV is prevalent in community-dwelling older adults and rarely causes severe disease. This suggests that watchful waiting, using a continuity of care approach to identify those who do need more intensive care is often justified when RSV is suspected in family practice.

INTRODUCTION

Respiratory Syncytial Virus (RSV) is responsible for a significant burden of disease among adults [1, 2]. RSV infections in adulthood are often milder than primary childhood infections, but can still cause severe respiratory disease [1, 3]. This is illustrated by the fact that the overwhelming majority of RSV mortality in industrialized countries occurs in those that are above 65 years of age [2, 4]. Studies in hospitalized patients and nursing home residents showed that severe RSV infection occurs in those who are older, have an immunodeficiency or underlying cardiopulmonary disease [1, 3, 5, 6]. Although RSV-awareness in medical settings is increasing, we still know surprisingly little about RSV-related disease in the general population. The only two cohort studies in older adults living in the community, so-called community-dwelling older adults, indicated an overall annual incidence of RSV infection of 3-7% in generally healthy older adults [1, 7]. However, both single-center studies were conducted 15 years ago and only the study by Falsey and colleagues [1] used both serology and PCR to confirm RSV infection. Therefore, the exact current burden of RSV in older adults in the general population is still uncertain. With a rising number of clinical trials investigating new therapeutics to treat or prevent RSV [8], relevant, precise and up-to-date evidence to inform about the value of these therapeutics in community-dwelling older adults is urgently required. To address this gap in evidence base, the REspiratory Syncytial virus Consortium in EUrope (RESCEU; www.resc-eu.org) project set out to assess the incidence and severity of RSV infection in community-dwelling older adults aged 60 years and above in its older adult cohort study.

METHODS

Study design

The RESCEU older adult study is an international, prospective, observational cohort study conducted in Antwerp (Belgium), Oxford (United Kingdom) and Utrecht (the Netherlands) across two consecutive RSV-seasons (2017-2018 and 2018-2019). Before the start of each RSV-season (October 1st – May 1st) an independent cohort of participants was recruited from 17 general practitioner's offices and followed up during one RSV-season.

Study population

Community-dwelling adults were eligible for inclusion if they were at least 60 years of age. Exclusion criteria were an estimated life expectancy of less than a year, chronic immunosuppressive illnesses or medication, and conditions such as severe dementia which would make it impossible to complete the necessary study procedures. The complete list of exclusion criteria can be found on Clinicaltrials.gov, identifier: NCT03621930 and in the study protocol [Supplemental file]. Eligible patients received an initial invitation letter by their general practitioner after which they were contacted by the study team for study recruitment [Supplemental file].

Study procedures

Between August and September a pre-season baseline home visit was performed during which patient characteristics were obtained and sampling was performed (amongst others, blood for RSV serology). Participants were contacted weekly by email or telephone during the RSV-season to ask for symptoms of acute respiratory tract infection (ARTI). ARTI was defined as the presence of one or more of the following symptoms for at least one day: cough, nasal congestion or discharge, wheezing or shortness of breath. Patients with ARTI were visited at home by the study team for viral testing within 72 hours after notification. RSV and influenza were tested within 24 hours after the home visit from the nasopharyngeal sample using a molecular point-of-case test (the Xpert® Xpress Flu/RSV assay (Cepheid, Sunnyvale, CA, USA)[9]. A second nasopharyngeal swab was collected for validation of RSV by qPCR. RSV-antibody titers (pre-F, post-F and neutralizing antibodies) were determined before and after the RSV-season [Supplemental file]. Vital signs (heart rate, respiratory rate, SpO_2 and temperature) were measured during the home visit and patients were instructed to complete a daily symptom log [Supplemental file], and noted doctors' visits and used medication during 28 days or for as long as symptoms were present. A post-season home visit was performed within two months after the RSV-

season during which clinical data and samples were collected similar to the baseline visit. Reported pneumonia and hospitalizations were verified by medical notes review.

Definitions

The primary outcome, RSV-illness, was defined as either a PCR-confirmed RSV-ARTI or a ≥4-fold increase in any RSV antibody titer post-season compared to baseline [Statistical Analysis Plan]. We distinguished within RSV-illness for RSV-ARTI (clinical ARTI, only based on PCR). Frailty was scored using the validated Groningen Frailty Indicator (GFI) questionnaire [10]. Higher scores represent increased frailty whereas the cut-off for frail is at ≥4. We classified ARTI for severity. Severe disease included hospitalization within 28 days after ARTI onset while moderate disease included any medical-attendance (except hospitalization) or new or increased used of inhaled respiratory medication, antibiotics, antivirals or corticosteroids. All other respiratory episodes were classified as mild disease.

Statistical analysis

Incidence of RSV-illness was calculated as the number of confirmed illnesses divided by the study population per season. ARTI incidence was calculated similarly for PCR-confirmed clinical infections. Confidence intervals were calculated using the Exact Clopper-Pearson method. Sensitivity analysis of the RSV incidence was performed to correct for uncertainty associated with the diagnostic tests. Test results were imputed in those with ARTI and a missed visit (no molecular test) or delayed testing (swab collected after seven days of symptom onset) if serology was not available. Subsequently, patients with a ≥2 to <4-fold rise in serum RSV antibodies (probable RSV) were added as cases to obtain the sensitivity estimates [Statistical Analysis Plan].

Second, patient characteristics, symptoms and vital signs, severity, and changes in frailty and cardiopulmonary status were compared between ARTI with different viral aetiology. We only compared PCR-confirmed ARTI since these could be directly linked to respiratory illness. Multivariable logistic regression analysis was performed to evaluate the prognostic performance (AUC) of symptoms for predicting RSV-ARTI. Clinically relevant symptoms (cough, dyspnoea, wheeze, phlegm and fever) were included in this model. Missing data was not imputed except for the sensitivity analysis. Available data from cases that were lost to follow-up during the study was used if permitted. All analyses were performed in R version 4.0.1 and the mice package was used for multiple imputation.

RESULTS

Study population

Out of 6398 invitations sent out by the general practitioners, we included 1040 participants (16%) [Figure 1]. 527 participated during the 2017-2018 season, and 513 participated during the 2018-2019 RSV-season [Table 1]. Participants in the second season were older, lived alone more frequently, had a higher prevalence of cardiac comorbidity and used more medication. Thirty-eight participants (3.7%) were lost to follow-up during the study including nine participants who died during the study [Figure 1]. No deaths were associated with respiratory infection. Participants lost to follow-up were older, had more comorbidity and were more often considered frail than those successfully followed up (data not shown).

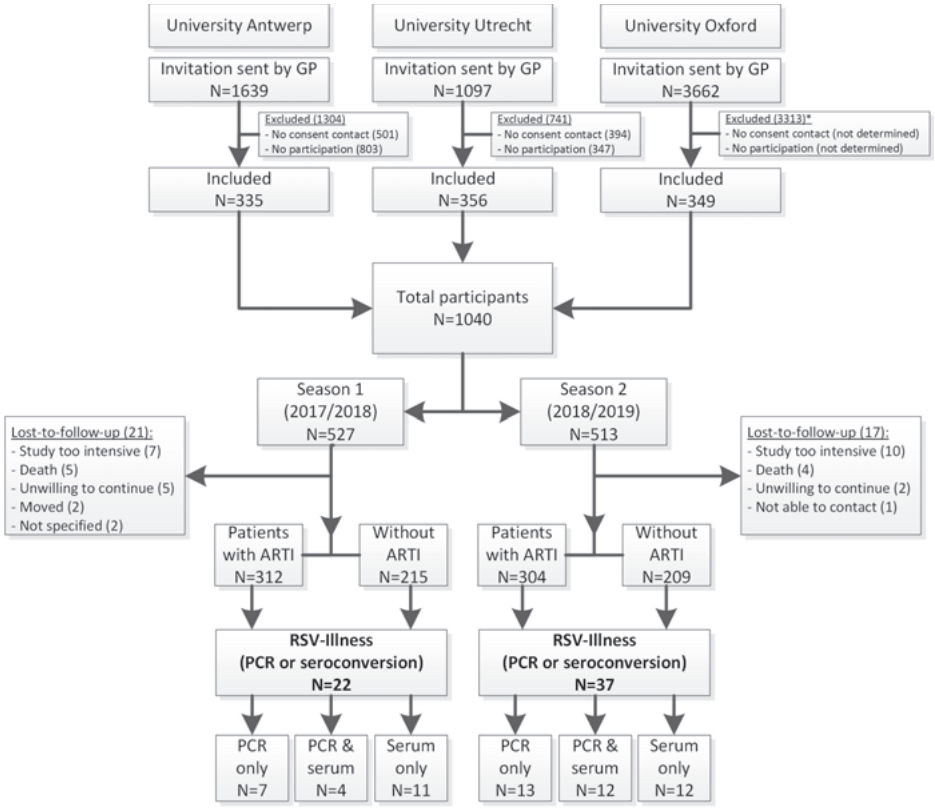


Figure 1. Recruitment, flow and outcomes in the older adult cohort study
ARTI: Acute Respiratory Tract Infection, PCR: Polymerase Chain Reaction. *Although precise numbers could not be determined, the majority (>80%) of non- inclusions did not actively return consent and were therefore never approached for recruitment in the study (opt-in procedure).

Acute respiratory tract infections

In total, 844 ARTIs were reported by 616/1040 participants (59%, range 1-5 episodes). Study team visits were performed in 95% (805/844) of ARTIs. Median time between onset of symptoms and the study visit was four days (range 0-33) days and 88% of tested ARTIs were visited within one week after onset of symptoms (78% in the first, 97% in the second season). 39/844 ARTIs in 39 individual patients were reported but were not tested ("missed visits"), most often because the study team was not notified until after the ARTI was resolved (N=31).

Table 1. Characteristics of study participants

	Total study population N = 1040	Season 2017-2018 N = 527	Season 2018-2019 N = 513
Study site:			
Belgium	335 (32%)	204 (39%)	131 (25%)
Netherlands	356 (34%)	148 (28%)	208 (41%)
United Kingdom	349 (34%)	175 (33%)	174 (34%)
Age:			
Years; median (range)	75 (60-100)	70 (60-95)	78 (60-100)
Age above 75	562 (54%)	174 (33%)	388 (76%)
Female sex	554 (54%)	268 (51%)	286 (56%)
Northwest European[a]	999 (97%)	515 (98%)	484 (94%)
Living situation:			
Living alone	338 (33%)	146 (28%)	192 (37%)
Living with partner	666 (64%)	363 (69%)	303 (59%)
Other	36 (3%)	18 (3%)	18 (4%)
High educational level[b]	394 (38%)	217 (41%)	177 (35%)
Comorbidity (any)	697 (67%)	316 (60%)	381 (75%)
Cardiovascular disease[c]	212 (21%)	78 (15%)	134 (26%)
Congestive Heart disease	11 (1%)	5 (1%)	6 (1%)
Lung disease[c]	120 (12%)	55 (10%)	65 (13%)
Asthma	54 (5%)	29 (6%)	25 (5%)
COPD	54 (5%)	22 (4%)	32 (6%)
Cardiovascular or lung disease[c]	307 (30%)	121 (23%)	186 (37%)
Diabetes[c]	80 (8%)	35 (7%)	45 (9%)
Allergies (any)[1]	276 (27%)	131 (25%)	145 (29%)
Hay fever	59 (6%)	23 (4%)	36 (7%)

Table 1. Continued

	Total study population N = 1040	Season 2017-2018 N = 527	Season 2018-2019 N = 513
House dust mite	32 (3%)	21 (4%)	11 (2%)
Polypharmacy (>4 medicines)	372 (36%)	165 (31%)	207 (40%)
Respiratory medication	174 (17%)	88 (17%)	86 (17%)
Pneumococcal vaccination[2]	118 (13%)	75 (16%)	43 (9%)
Influenza vaccination[3]	752 (76%)	359 (73%)	386 (80%)
Smoking status			
Current smoker	80 (8%)	42 (8%)	38 (7%)
Former smoker	409 (39%)	200 (38%)	209 (41%)
Alcohol status			
Current drinker (≥1 unit per week)	666 (64%)	349 (66%)	317 (62%)
Average consumption	1-7 units/week	1-7 units/week	1-7 units/week
Frailty[4]			
GFI score; median (range)	2 (0-12)	2 (0-12)	2 (0-12)
Frail (GFI score ≥4 points)	148 (15%)	70 (14%)	78 (17%)

Abbreviations: COPD = Chronic Obstructive Pulmonary Disease; GFI = Groningen Frailty indicator. [a] Born in one of the three participating countries or directly surrounding countries. [b] Defined as university of applied sciences or higher. [c] Cardiovascular comorbidity included all arrhythmias, structural heart diseases, angina and cardiac events such as infarction, percutaneous coronary intervention and bypass surgery. Hypertension was not included in this definition. Lung disease included asthma, COPD, chronic bronchitis and emphysema. Diabetes was defined as either type one or two or unspecified diabetes. Missing data <1% is not shown, if more than 1% is missing, the percentages are added as footnote. [1]missing N=20 (2%), [2]Missing N=95 (9%), [3]missing N=52 (5%), [4]missing N=78 (8%)

Incidence of RSV and influenza

RSV-illness, based on PCR or ≥4-fold seroconversion, was diagnosed in 59/1040 participants. We diagnosed 22/527 participants (4.2%, 95% CI 2.6-6.3%) in the first, and 37/513 (7.2%, 95% CI 5.5-10.2%) in the second RSV-season [Table 2]. RSV-illness was detected by PCR (20 cases), serology (23 cases) or both (16 cases) [Table 2]. Most RSV-illnesses identified only by serology did experience an ARTI during follow-up (16/23, 70%) which was either PCR-negative (20 ARTI in 13 patients) or were from a missed visit (3 patients) [Table S1-S3]. RSV-ARTI, based on PCR only, was diagnosed in 11/527 patients (2.1%, 95% CI 1.0-3.7%) in the first, and 25/513 (4.9%, 95% CI 3.2-7.1%) in the second RSV-season [Table 2, Figure 2]. Medically-attended RSV (MA-RSV) was seen in 4/527 (0.8%) patients in the first, and 7/513 (1.4%) patients in the second RSV-season. RSV B

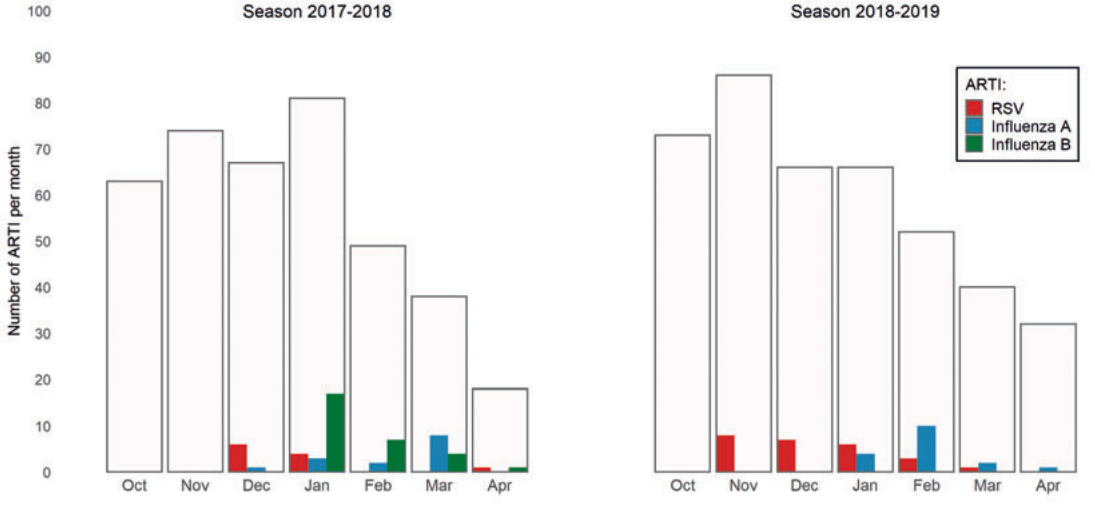
was most often detected (26/32 subtyped RSV-ARTI) during both seasons [Table S1]. No RSV reinfection or coinfections with influenza occurred. Sensitivity analyses showed an incidence of 8.0% (5.8–10.6%) in the first, and 9.9% (7.5-12.8%) in the second RSV-season [Supplemental file].

Influenza-ARTI, based on PCR only, was detected in 59 participants [Table S1]. Influenza A incidence was 2.7% (14/527) in the first season and 3.3% (17/513) in the second season. Influenza B was only detected in the first season in 5.5% (28/527) participants. RSV-ARTI incidence was lower compared to influenza-ARTI in the first season (1.9% versus 8.2%, respectively), but not in the second season (4.7% versus 3.3%) [Table S1]. Baseline characteristics were similar for patients with ARTI by different viral aetiologies [Table 3, Table S3].

Table 2. RSV infection

	2017-2018 (N = 527)		2018-2019 (N = 513)	
	Cases	% (95% CI)	Cases	% (95% CI)
RSV-illness[a]	22	4.2% (2.6- 6.3)	37	7.2% (5.5 - 10.2)
PCR positive[b]	11	2.1% (1.0-3.7)	25	4.9% (3.2-7.1)
Seroconversion[c]	15	2.8% (1.6-4.7)	24	4.7% (3.0-6.9)

[a] Either positive PCR or evidence of seroconversion [b] Based on positive PCR or POCT [c] based on ≥4-fold increase in any antibody titer.



ARTI Type	Oct-17	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18	Apr-18	-	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	Total
RSV A	0	0	2	3	0	0	0	-	0	0	1	0	0	0	0	6
RSV B	0	0	3	1	0	0	1	-	0	7	4	6	3	1	0	26
RSV unknown	0	0	1	0	0	0	0	-	0	1	2	0	0	0	0	4
Influenza A	0	0	1	3	2	8	0	-	0	0	0	4	10	2	1	31
Influenza B	0	0	0	17	7	4	1	-	0	0	0	0	0	0	0	29
Other ARTI	63	74	60	57	40	26	16	-	73	78	59	56	39	37	31	709
Total	63	74	67	81	49	38	18	-	73	86	66	66	52	40	32	805

Figure 2. Observed respiratory infections per study season

ARTI: Acute Respiratory Tract Infection. ARTI are ordered based on the date of the positive test. Only those with a result from molecular testing on nasopharyngeal swab are included in this figure and table. The white columns represent the total number of ARTI. Unknown RSV (n=4) were not subtyped since these cases were not tested by qPCR

Table 3. Characteristics of patients with PCR-confirmed ARTI

	RSV-ARTI patients N= 36	Influenza-ARTI patients N= 59	Other ARTI patients N= 477	Patients without ARTI N= 417
Age; median years [IQR]	75 [70-79]	71 [67–78]	75 [68-80]	76 [69-81]
Female sex	20 (56%)	30 (51%)	261 (55%)	216 (51%)
High educational level[a]	17 (47%)	28 (48%)	183 (38%)	154 (37%)
Comorbidity (any)	23 (64%)	37 (63%)	338 (71%)	268 (65%)
Cardiac disease[b]	7 (19%)	10 (17%)	103 (22%)	84 (20%)
Congestive heart disease	1 (3%)	1 (2%)	4 (1%)	5 (1%)
Lung disease[b]	5 (14%)	7 (12%)	63 (13%)	39 (9%)
Asthma	2 (6%)	5 (9%)	31 (7%)	16 (4%)
COPD	1 (3%)	3 (5%)	25 (5%)	20 (5%)
Diabetes[b]	2 (6%)	5 (9%)	51 (11%)	19 (5%)
Polypharmacy (≥4)	12 (33%)	17 (29%)	187 (39%)	136 (33%)
Respiratory medication	6 (17%)	13 (22%)	92 (19%)	48 (12%)
Previous influenza vaccination[1]	30 (86%)	46 (78%)	359 (78%)	278 (72%)
Previous pneumococcal vaccination[2]	4 (12%)	10 (20%)	55 (13%)	41 (10%)
Current smoker	3 (8%)	3 (5%)	29 (6%)	39 (9%)
Former smoker	14 (39%)	17 (29%)	206 (43%)	153 (37%)
Frailty[3]				
Frail baseline[c]	2 (6%)	6 (11%)	71 (16%)	60 (16%)
GFI score baseline; median [IQR]	1.5 [1-3]	2 [1-3]	2 [1-4]	2 [1-4]
GFI change over season; median [IQR]	0 [-1 – 1]	0 [-1 – 1]	0 [-1 – 1]	0 [-1 – 1]
Developed frailty	0 (0%)	3 (6%)	19 (5%)	15 (5%)
Lost frailty	1 (3%)	0 (0%)	36 (9%)	28 (9%)
Worsening of cardiorespiratory status[4]				
New lung disease	0 (0%)	0 (0%)	9 (2%)	3 (1%)
New cardiac disease	0 (0%)	1 (2%)	3 (1%)	1 (0.3%)
Increased respiratory medication	1 (3%)	3 (5%)	18 (4%)	8 (2%)

Abbreviations: IQR=interquartile range; GFI = Groningen Frailty indicator. 23 patients with only serologic evidence of RSV infection and 28 patients with a missed visit were excluded from this table. Three patients had separated RSV and influenza-ARTI during follow-up and were counted in both groups while one patient experienced two separate influenza B infections and was counted once [a] Defined as university of applied sciences or higher. [b] Cardiovascular comorbidity included all arrhythmias, structural heart diseases, angina and cardiac events such as infarction, percutaneous coronary intervention and bypass surgery. Hypertension was not included in this definition. Lung disease included asthma, COPD, chronic bronchitis and emphysema. Diabetes was defined as either type one or two or unspecified diabetes. [c] GFI score of ≥4 points. Missing data <1% is not shown, if more than 1% is missing, the percentages are added as footnote. [1]missing N=52 (5%), [2]missing N=95 (9%), [3]missing baseline N=78 (8%), missing end-of-season N=114 (11%), missing either N=180 (17%) [4]missing N=62.

Severity of infection

Severity was compared between 805 PCR-confirmed ARTI [Table 4]. Four ARTI episodes required hospitalization. All were PCR-negative for RSV (one was PCR-positive for influenza). There was no ARTI-related mortality. RSV-ARTI required less medical attendance (31% vs 60%, p=0.006) and fewer antibiotic prescriptions (6% vs 31%, p=0.004) compared to influenza-ARTI. Symptom duration for RSV-ARTI averaged 19 days and was significantly longer compared to other infections (19 vs 12 days, p=0.006), but similar to influenza-ARTI (19 versus 18 days, p=0.53). 22% of RSV-ARTI still had symptoms after 28 days. Similar results were observed for A and B subtypes of RSV and influenza [Table S4]. Another four patients were hospitalized from the 39 missed visits and had therefore no molecular test. No evidence of RSV infection was seen in three of these hospitalized patients of whom serology was available.

Table 4. Severity of PCR-confirmed ARTI episodes

	RSV-ARTI episodes N= 36	Influenza-ARTI episodes N= 60	Other ARTI episodes N= 690[a]
Median duration of symptoms [IQR]	19 [13-27]	18 [14- 22]	12 [8-21]**
Unresolved illness[b]	8 (22%)	9 (16%)	105 (17%)
Medication[c]	10 (28%)	26 (44%)	99 (15%)
Respiratory medication	9 (25%)	13 (22%)	68 (10%)*
Antibiotics	2 (6%)	18 (31%)**	49 (7%)
Antivirals	0 (0%)	2 (3%)	0 (0%)
Corticosteroids	0 (0%)	2 (3%)	9 (1%)
Medical attendance	11 (31%)	36 (60%)**	138 (20%)
Hospitalization	0 (0%)	1 (2%)	3 (0.4%)
Emergency department	0 (0%)	0 (0%)	1 (0.2%)
General practitioner visit	10 (28%)	32 (55%)*	122 (18%)
Telephone call to doctor	2 (6%)	3 (5%)	7 (1%)
LRTI[d]	0 (0%)	1 (2%)	3 (0.4%)
Death	0 (0%)	0 (0%)	0 (0%)
Severity classification			
Mild	22 (61%)	20 (33%)*	505 (75%)
Moderate	14 (39%)	39 (65%)*	169 (25%)
Severe	0 (0%)	1 (2%)	3 (0.4%)

Abbreviations: IQR=interquartile range; LRTI = Lower respiratory tract infection. Statistical significance compared to RSV-ARTI is indicated by the asterisks: *p-value<0.05 **p<0.01 ***p<0.001 (not indicated if non-significant). [a] 19 episodes with other infection but positive seroconversion for RSV and 39 missed visits were excluded from this table [b] Illness that persisted beyond the 28 diary days. [c] Enhanced use or newly prescribed inhaled respiratory medication, antibiotics, antivirals or corticosteroids. [d] clinically diagnosed or radiologically confirmed pneumonia.

Frailty and comorbidity

Groningen Frailty Indicator (GFI) scores were significantly higher at baseline in those with older age (p=0.001), with comorbidity (p<0.001), who lived alone (p=0.001), and who had a low educational level (p<0.001) (data not shown). Neither the GFI score at baseline nor age and comorbidity were associated with occurrence or severity of RSV-illness or RSV-ARTI [Table S5]. Neither RSV infection nor ARTI affected frailty or cardiopulmonary status in this generally healthy older adult population [Table 3].

Clinical symptoms

Diary information was available in 750/805 (93%) of ARTIs. Patients with RSV and influenza generally reported more symptoms compared to other ARTI [Table 5]. We observed substantial variation in symptomatology with little specificity for RSV or influenza. Multivariable modelling including cough, phlegm, dyspnoea, wheeze, and feeling feverish showed limited prognostic accuracy (AUC 0.66, 95% CI 0.59-0.74) (data not shown).

Table 5. Clinical symptoms of respiratory episodes

Patient reported symptoms [a]	RSV-ARTI episodes N= 36	Influenza-ARTI episodes N= 57	Other ARTI Episodes [b] N= 657
Rhinitis	36 (100%)	55 (96%)	624 (95%)
Cough	35 (97%)	55 (96%)	572 (87%)
Wheeze	16 (44%)	26 (46%)	223 (34%)
Phlegm	34 (94%)	52 (91%)	466 (71%)**
Dyspnea	24 (67%)	42 (74%)	309 (47%)*
Fever (measured ≥38°C)	2 (6%)	11 (19%)	26 (4%)
Feeling feverish	12 (33%)	37 (65%)**	191 (29%)
Headache	27 (75%)	45 (79%)	348 (53%)*
Myalgia	19 (53%)	41 (72%)	263 (40%)
Disturbed sleep	26 (72%)	51 (89%)*	440 (67%)
Feeling unwell	33 (91%)	56 (98%)	499 (76%)*
Disturbance in daily activity	27 (75%)	51 (89%)	348 (53%)**
Vital signs from home visit [c]			
Fever (measured ≥38°C)	2 (6%)	9 (16%)	13 (2%)
Respiratory rate >20/min	6 (17%)	8 (14%)	63 (10%)
Saturation SpO_2 < 95%	5 (14%)	10 (18%)	39 (6%)

Numbers represent respiratory episodes unless stated otherwise. Abbreviations: ARTI = acute respiratory tract infection. Statistical significance compared to RSV-ARTI is indicated by the asterisks: *P<0.05 **P<0.01 ***P<0.001 (not indicated if non-significant). [a] At least once during the respiratory infection based on the symptom diary [b] RSV and influenza negative infections based on PCR. [c] Measured by the study team.

DISCUSSION

In this study we found an annual incidence of RSV-illness of 4.2% and 7.2% in community-dwelling older adults in Europe. While prevalent, our study shows that most RSV infections were mild and did not require hospitalization or led to worsening of frailty or cardiopulmonary status. There were no RSV-associated deaths. To our knowledge, this is the first prospective multi-country observational cohort study providing estimates of the incidence and severity of RSV infection in community-dwelling older adults.

Our RSV incidence is in line with other prospective cohort studies in healthy community-dwelling older adults indicating an annual incidence of 1.6% to 7% [1, 7, 11-13]. Most comparable is the study by Falsey and colleagues [1]. Amongst other groups, they studied 608 older adults aged ≥65 years without disabling comorbidity during four RSV-seasons from 1999-2003. RSV incidence ranged from 3-7% between the seasons based on viral culture, PCR and serology. Nicholson and colleagues followed a cohort of 533 community-dwelling older adults and found an incidence of 3.2% although RSV diagnosis was solely based on serology [7]. This is in line with our serology-based incidences (2.8% and 4.7%). RSV vaccine trials typically showed lower estimates ranging from 1.6-3.4% in published [12, 13], and 1.97-4.9% in unpublished studies [11]. However, estimates were often based on single seasons, with different ARTI definitions, different participation criteria, and generally did not include serology.

RSV incidence in our study varied substantially per season although confidence intervals overlapped. Several factors may explain this difference. National surveillance indicated a higher RSV-peak in 2018-2019 in Belgium and the United Kingdom compared to 2017-2018 [14-18]. Second, delayed sampling was more common in our first season which might have resulted in misclassification by PCR [19]. Third, viral interference between RSV and influenza is suggested [20, 21]. The large 2017-2018 influenza B outbreak may have influenced the RSV-epidemic. Fourth, RSV incidence was higher in the second season when the cohort was significantly older and had more comorbidity compared to the first season. Although severity is associated with older age and comorbidity [1, 3, 22-24], RSV incidence was not associated with these factors in ours and other studies [22, 25].

While in-hospital RSV infections are associated with high morbidity and mortality [1, 6, 26], our results suggest that RSV infections in community-dwelling older adults are generally mild and require limited intervention. Although contrasting, this finding is

not unexpected since the lack of mortality [1], non-existent to very low hospitalization rates [1, 2] and a lower rate of doctor's visits and antibiotic prescriptions compared to influenza in this population was observed before [1]. Symptoms and duration of illness was comparable with influenza-ARTI, except for fever, which was more often seen in influenza-ARTI. This could have attributed to more doctor's visits and antibiotic prescriptions in our study. None of the clinical symptoms could distinguish RSV from all other ARTI without viral testing. Our findings suggest that watchful waiting, using a continuity of care approach to identify those who do need more intensive care is justified in case of suspected RSV infection in the community. Careful monitoring of patients with an increased risk of severe disease like those with cardiopulmonary comorbidity should be part of this approach.

The main strength of this study is that we are the first to provide burden estimates of RSV infection using both PCR and serology from a large community cohort of older adults in multiple European countries. Crucial in the study design was premorbid recruitment and prospective follow-up of a representative community population. Recruitment from general practitioners offices made it possible to study a generalizable community population. Without the need of medical attendance to trigger an ARTI home visit, there was no selection bias for viral testing based on disease severity. With intensive surveillance during multiple RSV-seasons we managed to visit 88% of infections within one week after onset of symptoms.

Regarding limitations, first, testing early in the course of infection is crucial in diagnosing RSV in older adults [19]. Delayed testing did occur, most often during the first season (22% versus 3% in the second season). More serology-confirmed cases were identified compared to PCR-confirmed cases in this first season which could reflect misclassification by PCR. Three patients had detectable RSV by qPCR but were below the predefined limits of detection excluding them as cases in our analyses. This could have underestimated RSV incidence. Second, 39 ARTI-episodes, including four hospitalizations, were missed and therefore not sampled. Three of these missed ARTI showed seroconversion of RSV-antibodies but none of the hospitalized patients did. Third, without acute and convalescent serum flanking illnesses we could not determine the fraction of symptomatic RSV because we were unable to directly link serologic responses to illnesses. Symptom and severity analyses were therefore limited to PCR-confirmed ARTI limiting the power of these analyses. Fourth, since we collected convalescent serum after the season, antibody

decay could have occurred between acute RSV infection and convalescent sampling [27]. This could have underestimated the incidence and could explain why 87% (27/31) of PCR-confirmed cases had a ≥2-fold increase in serum antibodies but just 52% (16/31) showed a ≥4-fold increase. Sensitivity analysis including cases with probable seroconversion showed a total incidence of 8.0% (+3.8%) in the first, and 9.9% (+2.7%) in the second season. These estimates provide the upper limit of RSV incidence that could have occurred in our study although this is speculative. Fifth, influenza was only confirmed with PCR and not serology. This has underestimated the incidence of influenza in our study [28] and limited comparisons between influenza and RSV to PCR-confirmed ARTI. Sixth, the cohort was too small and perhaps 'too healthy' to provide estimates about more severe complications such as hospitalizations or death although the fact that we did not observed any for RSV is reassuring. Seventh, we might have missed progression of frailty in any group due to the relatively healthy study population at the start of follow-up. Also, measurement at baseline and after the season could be too long to assess the short term impact of respiratory infection, or too short to assess long lasting increases in frailty. Eight, study visits and testing for RSV could have influenced health-care seeking behaviour. The proportion of MA-RSV was 31% which is in line with the 17-45% observed in similar studies [1, 7]. Last, selection bias could have occurred since 16% of those invited by their GP participated. However, the majority of non-inclusions were never contacted by the study team because of the way recruitment was organized and were not excluded based on unwillingness to participate or predefined criteria.

CONCLUSION

This well-powered prospective European cohort study showed that RSV is prevalent in community-dwelling older adults but rarely causes severe disease. This study confirms and updates estimates from earlier studies but also emphasizes the variability between seasons and importance of using different methods of RSV detection. This should help patient management in family practice when RSV is suspected, but also aid efforts to develop vaccines and therapeutics against RSV and guide implementation of preventive strategies, when RSV vaccines become available.

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SUPPLEMENTAL FILE:

Burden of respiratory syncytial virus infection in community-dwelling older adults in Europe (RESCEU): an international prospective cohort study

CONTENT:

Recruitment

Eligible adults were initially contacted by their general practitioner to inform them about the study by means of an invitation letter. In Belgium and the Netherlands an opt-out procedure was used in which patients were subsequently contacted by the study team unless they indicated they preferred not to be contacted. In the United Kingdom, an opt-in strategy was used in which participants were contacted upon active consent for contact following the initial GP invitation letter. Upon contact, patients were informed about the exact study procedures and in- and exclusion criteria were verified by telephone. Verbal consent was obtained to plan a baseline visit. Written informed consent was signed during the baseline visit in August-September each year. To ensure that all age groups were represented, it was indicated in the study protocol that half of the study population should be aged >75 years. This allowed the sites to deliberately recruit more older participants in the second season to ensure a good representation of all age groups as intended per protocol.

Sensitivity analyses

Sensitivity analyses included imputation of test results of six participants with a missed visit and 13 participants in whom viral testing was delayed. This showed an incidence of 4.4% (+0.2%) in the first, and 7.4% (+0.2%) in the second season. Subsequent addition of participants with probable seroconversion (≥2-fold increase in serum antibodies, n=83)

as RSV cases resulted in a total incidence of 8.0% (5.8–10.6%) in the first, and 9.9% (7.5-12.8%) in the second RSV-season.

Lab analyses

Sample collection

During the home visit two nasopharyngeal swabs were collected for viral diagnostics (FLOQSwab™, 3ml UTM Xpert viral transport medium, Copan diagnostics and MicroTest M4RT, Remel). RSV and Influenza were tested within 24 hours after the home visit from the nasopharyngeal sample using the Xpert® Xpress Flu/RSV assay (Cepheid, Sunnyvale, CA, USA)[9], a point of care qualitative real-time PCR. The second nasopharyngeal swab was stored at -80 degrees. After the second RSV-season these second nasopharyngeal samples were tested for RSV (including subtyping) using an in-house quantitative PCR (qPCR). RSV specific pre- and post-fusion as well as neutralizing antibodies in serum were measured at baseline and after the RSV-season. Serology analysis was not performed for influenza. Specification of the specific lab analyses is shown hereafter.

Polymerase Chain Reaction (PCR)

Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used to discriminate RSV A and RSV B subtypes. RSV A and RSV B RNAs extracted from the nasal-swabs are detected and quantified in a duplex RT-PCR format using specific amplification primers and fluorescent probes designed in the RSV N gene, encoding the RSV nucleocapsid protein. The process involves nucleic acids extraction, conversion of RNA to complementary deoxyribonucleic acid (DNA) by reverse transcription and detection by real-time PCR reaction using a calibration curve (absolute quantitation). The limit of detection (LOD) for RSV-A is 304 copies/ml of swab while for RSV-B the LOD is 475 copies/ml of swab. The RSV viral load is reported as copies of RSV RNA per mL of sample.

Serology

Pre-F ELISA:

Streptavidin ELISA plates are coated with biotinylated Pre-F protein [1]. Plates that contain a reference standard and those with the clinical samples are incubated for two hours. Subsequently, an HRP-conjugated mouse anti-human IgG(Fc) detection antibody is added and the antigen specific IgG binding is measured by a luminescent readout. From the mean of the duplicate measurement, Gen5 software is used to calculate the anti-RSV-

Pre-F antibody concentration, by referring the samples' luminescence to the 4-PL fit of the standard curve. The antibody concentration is reported in arbitrary EU/L. Lower limit of quantification (LLOQ) of the assay is 14.1 EU/L and upper limit of quantification (ULOQ) is 56224 EU/L.

Post-F ELISA:

Post-F protein coated ELISA plates containing a reference standard and clinical samples are incubated for two hours [1]. Subsequently, an HRP-conjugated mouse anti-human IgG(Fc) detection antibody is added and the antigen specific IgG binding is measured by a luminescence readout. From the mean of the duplicate measurement, Gen5 software is used to calculate the anti-RSV-Post-F antibody concentration, by referring the samples' luminescence to the 4-PL fit of the standard curve. The antibody concentration is reported in arbitrary EU/L. LLOQ of the assay is 6.6 EU/L and ULOQ is 44280 EU/L.

Neutralizing antibodies RSV-A2 µPRNT50:

Test samples were heat inactivated, initially diluted 1:50, and 2-fold serially diluted in Virus Growth Medium (DMEM-Glutamax, 2% of FBS and 1% penicillin streptomycin). Virus was diluted to obtain 150 PFUs/wells in VGM, and added in a 1:1 ratio to diluted serum. After incubation at 37°C for one hour, plates containing Vero cells were inoculated with this mixture, centrifuged for 10 minutes at 700 x g and incubated at 37°C for one hour. Inoculum was removed and the methylcellulose overlay (0.75% in 1X MEM-2% FBS-2% PS, 8 mM glutamine, 0.2% $NaHCO_3$) is added on wells and plates were incubated at 37°C, 5% CO2 for 40 hours (±2h). After fixation (cold acetone 85%, 4°C for 1h), plates were immunostained with anti-RSV mouse antibody (Abcam 24011) 1h30 at 37°C then goat anti-mouse IgG PE (Invitrogen P852) for 1h30 at 37°C.The plates were enumerated with an Ensight reader (Perkin Elmer) and titers were quantified as the reciprocal serum dilution to obtain 50% virus inhibition.

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Table S1: Respiratory tract infections and diagnostics

	2017-2018				2018-2019				
	NL	BEL	UK	Total	NL	BEL	UK	Total	Total
Participants	148	204	175	527	208	131	174	513	**1040**
ARTI Episodes	136	154	125	415	177	122	130	429	**844**
POCT (Cepheid Xpert) performed	133	133	124	390	177	108	130	415	**805**
Validation qPCR (in-house) performed	126	127	106	359	173	107	120	400	**759**
Serology assays (seroconversion) performed	124	185	142	451	163	116	140	419	**870**
RSV infection									
Molecular tests									
POCT (Cepheid Xpert)	5	3	2	10	8	10	6	24	**34**
PCR (qPCR in-house)	6	2	2	10	9	9	4	22	**32**
RSV A (based on qPCR)	2	2	1	5	0	0	1	1	**6**
RSV B (based on qPCR)	4	0	1	5	9	9	3	21	**26**
Any PCR (POCT or qPCR)	6	3	2	11	9	10	6	25	**36**
Serology									
Neutralizing antibodies (≥4 fold)	3	2	1	6	6	3	4	13	**19**
Pre-Fusion antibodies (≥4 fold)	3	3	3	9	11	4	5	20	**29**
Post-Fusion antibodies (≥4 fold)	3	3	5	11	8	3	5	16	**27**
Seroconversion (≥4 fold)	5	4	6	15	11	6	7	24	**39**
Probable seroconversion (≥2 <4 fold)	8	9	6	23	11	4	6	21	**44**
RSV outcomes									
RSV-illness*	8	6	8	22	15	12	10	37	**59**
RSV (sensitivity)**	13	14	14	41	23	14	13	50	**91**
Influenza infection									
Influenza A (POCT, Cepheid)	3	3	8	14	3	10	4	17	**31**
Influenza B (POCT, Cepheid)	10	12	7	29	0	0	0	0	**29**
Any influenza (POCT, Cepheid)	13	15	15	43	3	10	4	17	**60**
Other infection* **	113	115	107	335	163	88	120	371	**706**

NL = Netherlands, BEL = Belgium, UK = United Kingdom Numbers represent either number of cases or positive tests. *Primary outcome of either PCR positive RSV infection or ≥4-fold increase (seroconversion) in any RSV antibody. ** RSV based on positive PCR or seroconversion of ≥2-fold increase in any RSV antibody *** RSV and influenza negative by POCT and PCR.

Table S2. Characteristics of RSV patients

	PCR positive				PCR negative*		No PCR (No ARTI)	
	Seropositive (≥4-fold) N = 16	Probable seroconversion (≥2 <4-fold) N = 11	No seroconversion N = 4	No serum available N = 5	Seropositive (≥4-fold) N = 16	Probable seroconversion (≥2 <4-fold) N = 20	Seropositive (≥4-fold) N = 7	Probable seroconversion (≥2 <4-fold) N = 12
Age (years)ϕ	76 [67-89]	70 [64-82]	78 [66-82]	74 [63-86]	73 [62-82]	75 [61-88]	78 [73-84]	70 [61-95]
Cardiopulmonary disease	7 (44%)	2 (18%)	1 (25%)	2 (40%)	2 (13%)	8 (40%)	2 (29%)	2 (17%)
Number of infections	16	11	4	5	23	27	0	0
Missed visits (No PCR)	0	0	0	0	3	1	0	0
Medical attendance	5 (31%)	4 (36%)	0 (0%)	2 (40%)	4/23 (17%)	8/27 (30%)	-	-
Time onset disease until PCR sampling (days)ϕ	3 [2-6]	4 [2-7]	3 [2-4]	3 [2-7]	4 [1-10]	4 [1-9]	-	-
Delayed sampling (> 7 days)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3/20 (15%)	3/26 (12%)	-	-
Time ARTI until convalescence serology (weeks)ϕ	21 [9-29]	24 [6-27]	24 [20-28]	26 [21-29]	23 [3-36]	20 [4-34]	-	-

Values are numbers and percentage of cases with that characteristic unless otherwise indicated by the ϕ indicating median [range]. * Only those with serologic evidence of RSV are included in this table; Patients without a PCR but with a reported (missed) infection are included in these columns.

Table S3. Characteristics patients with RSV-illness compared to the total study population

	Total study population N = 1040	RSV-illness* N=59
Study site:		
Belgium	335 (32%)	23(38%)
Netherlands	356 (34%)	18 (31%)
United Kingdom	349 (34%)	18 (31%)
Age:		
Years median [range]	75 [60-100]	75 [62-89]
Age above 75	562 (54%)	33 (56%)
Female sex	554 (54%)	36 (61%)
Northwest European†	999 (97%)	56 (95%)
Living situation:		
Alone	341 (33%)	18 (31%)
Only adults in the household	667 (64%)	40 (68%)
Children in the household	32 (3%)	1 (1%)
High educational level‡	394 (38%)	27 (46%)
Comorbidity§		
Cardiovascular disease	212 (21%)	10 (17%)
Lung disease	120 (12%)	6 (10%)
Cardiovascular or lung disease	307 (30%)	16 (27%)
Diabetes	80 (8%)	3 (5%)
Allergies (any) [1]	276 (27%)	15 (26%)
Hay fever	59 (6%)	4 (7%)
House dust mite	32 (3%)	0 (0%)
Respiratory medication	174 (17%)	9 (16%)
Polypharmacy (>4 medicines)	372 (36%)	19 (32%)
Pneumococcal vaccination[2]	118 (13%)	10 (18%)
Influenza vaccination[3]	752 (76%)	47 (81%)
Smoking status		
Current smoker	80 (8%)	7 (12%)
Former smoker	409 (39%)	22 (37%)
Alcohol status		
Current drinker	666 (64%)	30 (53%)
Average amount (mode)	1-7 glasses/week	1-7 glasses/ week
Frailty[4]		
GFI score median [range]	2 [0-12]	2 [0-7]
Frail (score > 4 points)	148 (15%)	6 (10%)

Values are numbers and percentage of cases with that characteristic unless otherwise indicated * Based on positive PCR at the moment of acute infection or seroconversion ≥4-fold over baseline. †Defined as university of applied sciences or higher. ǂ Groningen Frailty Indicator (GFI) score of ≥4 points. Missing data <1% is not shown, if more than 1% is missing, the percentages are added as footnote. [1]missing N=52 (5%), [2]missing N=95 (9%), [3]missing baseline N=78 (8%), missing end-of-season N=114 (11%), missing either N=180 (17%) [4]missing N=62.

Table S4. Severity of ARTI specified by RSV and influenza subtypes

	RSV-A N= 6	RSV-B N= 26	Influenza-A N=31	Influenza-B N= 29
Duration of symptoms; median [IQR]	10 [8-18]	19 [13-27]	18 [14-22]	17 [14-25]
Unresolved illness[a]	1 (17%)	5 (19%)	5 (19%)	4 (14%)
Medication[b]	3 (50%)	4 (15%)	13 (43%)	13 (45%)
Respiratory medication	3 (50%)	3 (12%)	7 (23%)	6 (21%)
Antibiotics	0 (0%)	1 (4%)	9 (30%)	9 (31%)
Antivirals	0 (0%)	0 (0%)	1 (3%)	1 (3%)
Corticosteroids	0 (0%)	0 (0%)	1 (3%)	1 (3%)
Medical attendance	1 (17%)	8 (31%)	19 (61%)	17 (59%)
Hospitalization	0 (0%)	0 (0%)	1 (3%)	0 (0%)
Emergency department	0 (0%)	0 (0%)	0 (0%)	0 (0%)
General practitioner visit	1 (17%)	7 (27%)	16 (55%)	16 (55%)
Telephone call to doctor	0 (0%)	1 (4%)	2 (7%)	1 (3%)
LRTI[c]	0 (0%)	0 (0%)	0 (0%)	1 (3%)
Death	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Severity classification				
Mild	3 (50%)	18 (69%)	11 (36%)	9 (31%)
Moderate	3 (50%)	8 (31%)	17 (55%)	19 (66%)
Severe	0 (0%)	0 (0%)	3 (10%)	1 (3%)

No statistical analysis has been done on these subgroups because of low numbers. Abbreviations: IQR=interquartile range; LRTI = Lower respiratory tract infection. 4 RSV cases had unknown subtyping since they were only tested using the POCT and were therefore excluded from this table. [a] Illness that persisted beyond the 28 diary days. [b] Enhanced use or newly prescribed inhaled respiratory medication, antibiotics, antivirals or corticosteroids. [c] clinically diagnosed or radiologically confirmed pneumonia.

Table S5. Risk groups and severity of PCR confirmed RSV-ARTI

		Duration of symptoms median days(range)	Medical attendance	Medication[a]
Cardiopulmonary Comorbidity[b]	Yes (n=12)	19 (6-28)	5 (42%)	4 (33%)
	No (n=24)	20 (4-28)	6 (25%)	6 (25%)
Cardiac comorbity	Yes (n=7)	19 (13-28)	3 (43%)	2 (29%)
	No (n=29)	20 (4-28)	8 (28%)	8 (28%)
Lung comorbidity	Yes (n=5)	20 (6-28)	2 (40%)	2 (40%)
	No (n=31)	19 (4-28)	9 (29%)	8 (26%)
Old age (>75 years)	Yes (n=20)	20 (7-28)	5 (25%)	5 (25%)
	No (n=16)	19 (4-28)	6 (38%)	5 (31%)
Frail (GFI ≥4)	Yes (n=2)	18 (8-28)	0 (0%)	2 (100%)
	No (n=32)	19 (4-28)	10 (31%)	8 (25%)

None of the differences were statistically significant at p-value<0.05. [a] Enhanced use or newly prescribed inhaled respiratory medication, antibiotics, antivirals or corticosteroids. [b] Either cardiac or lung comorbidity. Cardiac comorbidity included all arrhythmias, structural heart diseases, angina and cardiac events such as infarction, percutaneous coronary intervention and bypass surgery. Hypertension was not included in this definition. Lung disease included asthma, COPD, chronic bronchitis and emphysema.

Supplement: Diary Questionnaire

WEEK 1; symptoms each day
For each day, please give every symptom a score from 0 to 6. If you score 0 for all symptoms for two consecutive days, complete the weekly questions for week 1.

Score	Severity of symptom:
0	Normal/not affected
1	Very little problem
2	Slight problem
3	Moderately bad
4	Bad
5	Very bad
6	As bad as it could be

Symptoms	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Cough							
Phlegm (coughing up slime)							
Shortness of breath							
Wheeze (during breathing out)							
Blocked/runny nose							
Muscle Ache							
Headache							
Disturbed sleep							
Feeling generally unwell							
Interference with normal activities/ work							
Interference with social activities							

Please score your temperature daily;

Score	**Severity of symptom**
.....°C	If measured, please write down the degrees Celsius
0	Not measured but does not feel warm/feverish
1	Not measured but does feel warm/ feverish

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Temperature							

CHAPTER 6

Contact with young children increases the risk of respiratory infection in older adults in Europe – the RESCEU study.

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Unpublished

ABSTRACT

Objectives

Knowledge about how older adults get a respiratory infection is limited but crucial for planning preventive strategies. We aimed to determine how contact with young children living outside of the household affects the risk of acute respiratory tract infections (ARTI) in community-dwelling older adults.

Methods

This study is part of the European RESCEU older adult study. Weekly surveillance was performed to detect ARTI throughout two winter seasons (2017-2018, 2018-2019). Child exposure was assessed at baseline and was defined as having contact with children under age five who live outside of the household. The average attributable fraction was calculated to determine the fraction of ARTI that could be explained by exposure to these children.

Results

We prospectively established that 597/1006 (59%) participants had at least one ARTI during follow-up (including 35 RSV-ARTI and 58 influenza-ARTI). Child exposure increased the risk of all-cause ARTI (aOR 1.58 95%CI 1.21-2.08, p=0.001). This risk was highest in those with the most frequent contact (aOR 1.80 95%CI 1.23-2.63, p=0.003). The average attributable fraction of child exposure explaining ARTI was 10% (95% CI 5-15%).

Conclusion

One out of ten ARTI in community-dwelling older adults is attributable to exposure to preschool children living outside of the household.

INTRODUCTION

Acute respiratory tract infections (ARTI) are the leading cause of disease worldwide with an estimated annual incidence of 17.2 billion upper ARTI and 291 million lower ARTI [1]. The incidence of ARTI is highest in childhood and decreases with older age [2]. However, most severe disease occurs in the extremes of the age spectrum [3]. This is illustrated by the fact that 45% of all worldwide deaths due to lower ARTI occur in adults older than 70 years [3]. It is therefore important to protect this vulnerable older age group and decrease their risk of getting infected. We are aware that young children are a reservoir of (viral) ARTI and their role in introducing ARTI into their households has been established [4-6]. Contact with children increased the risk of ARTI in the adult population in other studies [7-9]. But despite many transmission studies in various settings [4-6, 10-19], the role of young children in the occurrence of ARTI in older adults that live outside of the child's household is not well known. Since the majority of older adults live independently of children in Europe and North-America [20], understanding the source of infection and the transmission patterns in this setting is fundamental to determine how preventive strategies should be deployed. The recent pandemic of SARS-CoV-2 underlines once more the necessity of having a thorough understanding of how social contacts drive the risk of ARTI in this vulnerable population in order to determine the impact of preventive strategies such as which isolation measures should be taken. In this study we aimed to investigate the contribution of child exposure to ARTI in community-dwelling older adults.

METHODS

Participants of the prospective REspiratory Syncytial virus Consortium Europe (RESCEU) older adult cohort study were analysed. The RESCEU study was performed to investigate the burden of disease of RSV in community-dwelling older adults. The study design and data collection have been described previously [33]. In summary, the RESCEU older adult study is a European multicentre, prospective, observational cohort study conducted across two consecutive RSV seasons (2017-2018 and 2018-2019) in the Netherlands, Belgium and the United Kingdom. In each season a cohort of community-dwelling adults of at least 60 years of age was recruited from 17 general practitioners offices before the start of the RSV season (defined 1st October – 1st May), and followed up during one RSV season. GP offices were located in urban and suburban areas. More detailed information about the RESCEU older adult study can be found at Clinicaltrials.gov, identifier: NCT03621930.

ARTI

Between October 1st and May 1st, the participants were contacted weekly by email or telephone by the study team to ask for presence of respiratory symptoms. If one or more of the following acute respiratory tract infection (ARTI) symptoms were present for at least one day a home visit was scheduled within 72 hours: nasal congestion or discharge, cough, wheezing or shortness of breath. During the home visit two nasopharyngeal swabs were collected for viral diagnostics (FLOQSwab™, 3ml UTM Xpert viral transport medium, Copan diagnostics and MicroTest M4RT, Remel). RSV and Influenza were tested within 24 hours after collection using a point-of-care qualitative PCR (Xpert® Xpress Flu/RSV, Cepheid, Sunnyvale, CA, USA)[21]. RSV was also validated in the second nasopharyngeal sample using quantitative PCR (qPCR) after the study. We stratified for all-cause ARTI, RSV-ARTI and influenza-ARTI.

Exposure

Several exposure variables were measured by questionnaire during a baseline home visit before the start of the RSV season (August/September). These included how often participants had contact with children below the age of five that were living outside of the subject's household. Exposure to children was stratified as having frequent (weekly), infrequent (less than weekly), or no contact at all, irrespective of the number of children they had contact with. Additionally, the number of human contacts during the past 24 hours as a proxy of average daily contacts, employment status and household composition were recorded. We defined households as those living alone and those

living with a partner. Participants that lived together with children were excluded from the analysis. We calculated ARTI attack rates in those living alone and those living with a partner using a subcohort of participants that lived together and participated both in the study. The secondary attack rate (SAR), i.e. the rate of those that are infected by an infected household partner, was calculated in this subcohort. Details about the estimation of the attack rates are provided in the supplemental file.

Statistical analysis

Univariate logistic regression was used to approximate the risk of all-cause ARTI for individual risk factors. Subsequently, all exposure variables with a univariate p-value <0.10 were included in a multivariable logistic regression model which was adjusted for confounders. Potential confounders for the association between exposure variables and ARTI were based on literature and selected based on the highest correlations with the aforementioned exposure variables observed in a correlation matrix. No variable selection was performed and adjusted estimates were obtained from the full model. Similarly, models were developed for RSV-ARTI and influenza-ARTI although these were considered explorative because of limited power due to the low number of cases. In these explorative models single univariate predictors ($p<0.10$) were included in the multivariable model together with a fixed set of confounders including age, frailty score and comorbidity.

We estimated the contribution of child exposure to the occurrence of ARTI by calculating the population-attributable risk (PAR) and average attributable fraction. Both metrics combine the effect size of a risk factor with its prevalence in the studied population to provide an estimate of the fraction of disease that could be explained by that risk factor. The PAR was calculated using the unadjusted relative risk (RR) and the prevalence of exposure among cases (P_e) using the following formula: P_e (RR-1) / 1+P_e (RR-1). We thereafter calculated the average attributable fraction which was corrected for other exposure variables and confounders [22]. The average attributable fraction is calculated by modelling disease occurrence as a function of predictors (of which child exposure was of interest). By removing variables from the model one can estimate that variables' contribution to the disease occurrence. Averaging all attributable fractions from every possible permutation for which different predictors could be excluded from the model provides the average attributable fraction per included predictor. Details about the average attributable fraction can be found in the supplemental file. No imputation of

missing data was performed and all analyses were performed in R version 4.0.1. The Averisk package was used for calculating the average attributable risk [22].

RESULTS

1040 participants were included in the RESCEU study of whom 1006 (97%) lived in a household without young children. 597/1006 (59%) experienced at least one ARTI during the course of the study (range 1-5). Eight patients (0.9% of ARTI) were hospitalized while 211 had an outpatient visit (26% of ARTI). In total 822 ARTIs were reported in these 597 patients of which 783 were visited by the study team for diagnostic testing at the moment of infection. RSV-ARTI was detected by PCR in 35/783 (4.5%) patients while 58/783 (7.4%) patients had a PCR confirmed influenza-ARTI. No RSV-influenza co-infections occurred. Most respiratory infections had negative tests for RSV and influenza ("other ARTI"). The characteristics of study participants are displayed in Table 1. The subcohort of participants that lived with their partner and participated together in the study included 316 individuals (158 households). In total, 39 missed visits in 39 patients were registered (i.e. an infection was reported but no visit was performed). Missed study visits most often occurred because the infection was only mentioned after it was resolved or because participants were abroad. Characteristics of the total study population and the household subcohort are displayed in supplemental Table S1.

Child exposure

Any exposure to children below the age of five living outside of the household was reported in 51% (502/1006) of participants. Patients with influenza infection most often reported having contact with children (60%, 33/57) which was lowest in those without any respiratory infection (43%, 174/409). Frequent child exposure (weekly) was most often seen in RSV positive patients (31%, 11/35) and was again lowest in those without ARTI (15%, 61/409) [Table 1].

Table 1. Characteristics of study participants with and without a respiratory infection

	No ARTI	ARTI		
Demographics	N = 409	All-cause N = 597 [a]	RSV N= 35	Influenza N = 57
Age [median, IQR]	76 [70-81]	75 [68-80]	75 [70-80]	71 [67-78]
Gender female	212 (52%)	322 (54%)	20 (57%)	29 (51%)
Comorbidity (any)	269 (66%)	411 (69%)	22 (63%)	37 (65%)
Cardiovascular	86 (21%)	123 (21%)	7 (20%)	10 (18%)
Pulmonary	38 (9%)	78 (13%)	5 (14%)	7 (12%)
Diabetes	18 (4%)	60 (10%)	2 (6%)	5 (9%)
Frail [1,b]	59 (16%)	83 (15%)	2 (6%)	6 (11%)
Influenza vaccination[2]	274 (72%)	458 (80%)	29 (85%)	45 (79%)
Pneumococcal vaccination[3]	41 (11%)	72 (14%)	4 (12%)	10 (21%)
Smoking	39 (10%)	40 (7%)	3 (9%)	3 (5%)
Household smoke exposure	50 (12%)	62 (11%)	3 (9%)	5 (9%)
Allergic (any)[4]	97 (24%)	168 (29%)	9 (26%)	15 (27%)
Hay fever	22 (6%)	32 (6%)	3 (9%)	2 (4%)
House-dust mite	8 (2%)	23 (4%)	0 (0%)	4 (7%)
Exposure				
Exposure to children < 5 years[5]				
Any	174 (43%)	328 (55%)	18 (51%)	33 (60%)
Infrequent (< weekly)	113 (28%)	197 (33%)	7 (20%)	22 (40%)
Frequent (weekly)	61 (15%)	131 (22%)	11 (31%)	11 (20%)
Household composition				
Living alone	140 (34%)	198 (32%)	6 (17%)	18 (32%)
Living with partner	267 (65%)	395 (64%)	28 (80%)	39 (68%)
Other (adults only)	2 (1%)	4 (1%)	1 (2%)	0 (0%)
Daily contacts [median, IQR]	5 [3-10]	5 [2-10]	5 [4-9]	6 [3-15]
Employed[6]	49 (13%)	71 (12%)	6 (18%)	8 (15%)

Abbreviations: ARTI = acute respiratory tract infection, IQR = interquartile range. Values are numbers and percentage of cases unless otherwise indicated. [a] Including the 35 RSV and 57 influenza patients [b] Scored using the Groningen Frailty Indicator (GFI) questionnaire, a 15-item validated screening instrument to determine the level of frailty in adults, the cut-off for frail is at four points [23]. Missing data <1% is not shown, if more than 1% is missing, the percentages are added as footnote. [1]missing N=74 (7%), [2]missing N=52 (5%), [3]missing N= 89 (9%), [4]missing N=19 (2%), [5] missing N=11 (1%), [6] missing N = 38 (4%).

Child exposure and ARTI

Child exposure was an independent risk factor for all-cause ARTI in multivariable regression analysis adjusted for age, frailty score and comorbidity which showed an adjusted OR of 1.58(95% CI 1.21-2.08, p=0.001). A dose-dependent effect towards a higher risk was observed in those with more frequent contact [Table 2]. Exposure to young children did not significantly affect the risk for RSV or influenza infection. The average attributable fraction adjusted for confounding and other exposure variables indicated that child exposure explained 10% (95% CI 5-15%) of all ARTI [Table 3]. None

of the other variables included in the model were statistically significant based on the confidence intervals.

Other exposure

None of the exposure variables beside child contact were significant for ARTI. For RSV-ARTI the risk was significantly higher in those with a partner that was infected with RSV (aOR 4.81, 95% CI 1.18-19.6) [Table 2]. The secondary attack rate (SAR) for RSV was also significantly higher compared to the population risk (21.3% versus 3.6%, respectively) [Table 3]. A similar trend was seen for influenza (18.8% versus 5.9%, p=0.18).

Table 2. Regression analysis of exposure variables

	All-cause ARTI (n=597)		RSV (n=35)		Influenza (n=57)	
Risk factor	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
Any child exposure	1.64 (1.3-2.1)***	1.58 (1.2-2.1)**	1.05 (0.5-2.1)	-	1.51 (0.9-2.7)	-
Infrequent [a] (< weekly)	1.51 (1.1–2.0)*	1.48 (1.1-2.0)*	0.65 (0.3-1.6)	-	1.65 (0.9–3.0)	-
Frequent [b] (weekly)	1.86 (1.3-2.7)**	1.80 (1.2-2.6)**	1.69 (0.8 – 3.7)	-	1.30 (0.6-2.7)	-
Living with partner [c]	0.99 (0.8-1.3)	-	2.31 (1.1-5.8)*	2.15 (0.9-5.2) ±	1.13 (0.7-2.0)	-
Infected partner	NA [d]	-	5.18 (1.1-19.0)*	4.81 (1.2-19.6)*	3.33 (0.7-11.5)±	2.57 (0.7-10.2)
Employed	0.97 (0.7-1.4)	-	1.57 (0.6-3.7)	-	1.09 (0.5-2.2)	-
Number of daily contacts	1.00 (0.99-1.01)	-	1.01 (0.98-1.02)	-	1.01 (0.99-1.02)	-

Abbreviations: ARTI = acute respiratory tract infection; Crude OR = univariate regression analysis; aOR = adjusted odds ratio corrected for age, Groningen Frailty Indicator score and comorbidity only in those with an univariate association p<0.10. ±p-value<0.10 *p-value <0.05 **p-value <0.01 ***p-value <0.001. Significant values P<0.05 are shown in bold. [a] Infrequent child exposure compared to never. [b] Frequent child exposure compared to never. [c] Compared to those living alone. [d] Not determined since patients with multiple infections could not be classified for a single disease and exposure status in this analysis (i.e. one participant could have been an index case while also been exposed to an infected partner during a separate infection).

Table 3. Average attributable fractions explaining ARTI occurrence.

Variables	RR	Prevalence among ARTI (%)	PAR [a]	Average attributable fraction (95% CI) [b]
Child exposure (Yes/No)	**1.24**	**55%**	**10.5%**	**10.0% (5-15%)**
Employed	1.00	13%	0%	0% (-3 - 2%)
Living with a partner	1.05	65%	2.8%	0.5% (-7 – 8%)
Number of contacts [c]	0.97	76%	-2.2%	-3.7% (-14 – 7%)
Age >75 years	0.90	53%	-5.7%	-4.5% (-11 – 2%)
Comorbidity	1.03	70%	2.3%	5.4% (-3 – 13%)
Frail	0.95	15%	-0.7%	-0.6% (-3 – 2%)
Female sex	1.03	54%	1.4%	1.6% (-5 – 8%)
High educational level	1.06	42%	2.3%	2.1% (-3 – 7%)

RR = relative risk, PAR population attributable risk. [a] calculated as: P_e (RR-1) / 1+P_e (RR-1) in which P_e is the prevalence of exposure among cases. The PAR is based on a crude relative risk and is therefore not corrected for correlations or confounding. [b] Calculated with Averisk package, all variables in the table were included in the model to correct for confounding and correlations [c] dichotomized as >2 contacts per day from number of daily contacts.

Table 4. Attack rates of ARTI

All-cause ARTI (at least one)	Cases	Denominator [a]	Attack rate	95% CI
Total study population	597	1006	59.3%	56.3-62.3%
In those living alone	198	338	58.6%	53.3-63.7%
In those living with partner [b]	189	316	59.8%	54.3-65.1%
Secondary cases (SAR)	40	155	25.8%	19.6-33.2%
RSV-ARTI				
Total study population	35	968	3.6%	2.6 - 5.0%
In those living alone	6	322	1.9%	0.9 – 4.0%
In those living with partner [b]	18	292	6.2%	3.9 – 9.5%
Secondary cases (SAR)	3	14	21.4%	7.6 – 47.6%
Influenza-ARTI				
Total study population	57	967	5.9%	4.6 – 7.6%
In those living alone	18	322	5.6%	3.6 – 8.7%
In those living with partner [b]	21	288	7.3%	4.8 – 10.9%
Secondary cases (SAR)	3	16 [c]	18.8%	7.5 – 43.0%

Abbreviations: ARTI = acute respiratory tract infection, SAR = Secondary attack rates, the proportion of secondary cases occurring while the index case still experienced symptoms or within seven days after the primary case is recovered, divided by the total number of exposed household contacts. [a] Denominators for specific ARTI vary because of excluded missing visits. [b] Participants from the household cohort are used for these estimations. [c] Two patients had onset of influenza on the same date and were considered a co-primary case therefore 16/18 index cases exposed their partner to influenza.

DISCUSSION

We investigated how contact with young children living outside of the household affected the risk of ARTI in older adults living in the community. We showed that contact with young children increased the risk of all-cause ARTI in a dose-dependent manner and that child exposure explained 10% of all ARTI in the community-dwelling older adult population.

No studies to date have determined the direct transmission dynamics between young children and elderly living outside of the child's household. The role of young children in adult ARTI has been pointed out for community-acquired pneumonia in adults and RSV in patients with COPD [7, 24-26]. In another study transmission of respiratory disease and carriage of Streptococcus pneumoniae was highest when interpersonal contact was physical and extended [27]. Additionally, they showed that contact from and with children aged <10 years involved proportionally more physical contact than contacts between older children and adults [27]. Importantly, this effect was not observed for short (below five minutes) 'casual' contacts. We hypothesize that transmission is likely to occur when grandparents babysit their grandchildren since contact is both extended and will involve physical contact. Patients with RSV infection in our study reported the highest proportion of frequent child exposure. Although we suspected to find an association between child exposure and RSV infection in our population, our study was not powered to show virus-specific associations.

Another clue towards the pivotal role of children in the spread of respiratory disease in the population comes from the experience with mass vaccination of school-aged children for influenza [8, 28-31]. The Japanese program that spanned over three decades resulted in a significant decrease of excess mortality from pneumonia, influenza and all-cause mortality in all age groups [8]. More recent experiences from introduction of paediatric influenza vaccination in the United Kingdom indicated similar population benefits while also being cost-efficient [28-30]. They concluded that the most efficient way of reducing overall influenza-attributable morbidity and mortality appears to be to target the key spreaders, i.e. the children [28]. The best evidence comes from a randomized trial in which children aged 36 months – 15 years in Hutterite colonies in North-America were cluster-randomized to receive either influenza immunization or hepatitis A vaccination [31]. A significant protective effect of influenza vaccination was observed for all community members in the intervention group [31].

The major strength of our study is the prospective follow-up in a large cohort of community-dwelling older adults. Participants were recruited before onset of ARTI in contrast to studies that use a case-ascertained design in which participants are only recruited upon medical attendance. Cases in these studies are likely to have been biased towards individuals with more severe symptoms that required a doctor's visit. More severe disease may affect the generalizability of risk factor analysis. Additionally, by calculating the average attributable fraction we were not only able to determine the effect size of child exposure on the individual level, but also showed how much of the ARTI risk could be attributed to this risk factor. Last, co-participation of 316 older adult life partners in 158 households provided a unique opportunity to analyse infection rates in small households with only older adults.

There are also limitations to our study. First, exposure was assessed at baseline and was not verified at the moment of acute infection. While we quantified the average frequency of contact with young children, we did not prospectively log these contacts nor did we specify them for duration and setting. Also, the average contacts per day were based on one previous day and could therefore be imprecise. The next steps in understanding the role of infants as a source of infection in older adults are transmission studies performed upon contact at the homes of older adults. Second, we assume that the underlying mechanism of the association is based on viral transmission from children to older adults. Since not all ARTI have a viral aetiology [32], we may have underestimated the effect size. We could only differentiate for RSV and influenza but were underpowered to robustly study these two specific viral pathogens. We expect transmission of RSV and influenza from young infants to older adults since these pathogens are prevalent in childhood. Third, our results may not be generalizable to those that live in nursing homes since we studied community-dwelling older adults and contact patterns might differ. Last, still a large part of disease occurrence was not explained warranting further research for the risk of infection because of contact with other age groups and for other risk factors.

These findings are important for planning preventive strategies in the elderly population. Restricting contact with young children may be used to decrease the burden of ARTI in the elderly population. While application of this restrictive measure might be feasible in times of epidemics or in very frail patients, there are obvious social and economic issues involved with sustaining these measures on a regular basis. Paediatric vaccination for respiratory pathogens may indirectly diminish the ARTI burden in older adults as

discussed for influenza. Regional variance in household compositions (e.g. children and older adults living together) can affect effectivity and feasibility of preventive measures [20]. Paediatric vaccination may be more beneficial in regions where children and elderly more often share households. These types of shared households will conversely make social distancing strategies more difficult to achieve. A thorough understanding of social dynamics and regional differences is therefore crucial to tailor protective strategies.

CONCLUSION

We show that the risk of ARTI in older adults living in the community is increased because of contact with young children that do not live in the same household. More frequent contact was associated with a higher risk of ARTI and 10% of all ARTI could be attributed to contact with young children.

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SUPPLEMENTAL FILE:

Contact with young children increases the risk of respiratory infection in older adults in Europe – the RESCEU study.

Methods for estimation of attack rates in RESCEU

Population attack rates for all-cause ARTI and PCR-confirmed RSV-ARTI and influenza-ARTI were compared to attack rates in those living alone and those that lived with a partner. The population attack rate was defined as the total number of (specific) ARTI divided by the total study population. The attack rates for those living alone was the number of (specific) ARTI divided by those that lived alone. To investigate the attack rates in those living with a partner, we analyzed participants in a subcohort of RESCEU who lived in the same household and both participated in the study. The attack rate in those that lived with a partner was calculated as the number of (specific) ARTI in divided by all household members in this household subcohort. Estimates were calculated per person (number of cases / number of participants).

Subsequently, we used the household subcohort to define the risk of secondary infection after being exposed to an infected household member. A primary or index case was defined as anyone who developed an ARTI while there were no ARTI cases in the household in the past seven days before their symptom onset (based on the 95% upper limits of incubation periods for RSV and influenza[1]). Any healthy household member that was exposed to an index case with active respiratory symptoms was classified as an exposed household member. A secondary case was defined as an exposed household member that developed an ARTI caused by the same pathogen while the index patient was still sick or within seven days after the symptoms ceased [1]. The secondary attack rate (SAR), i.e. the risk of being infected after being exposed to an infected household member, was calculated as the number of secondary cases divided by the total number of exposed household members. The serial interval was calculated as the difference in the dates of onset of symptoms between the index case and the secondary case. The serial interval had to be ≥1 day to be classified as a secondary case. If two cases had the same onset date, the infection was considered to have a common source outside of the household and they were classified as a co-primary case. For all attack rate estimates we calculated 95% confidence intervals using the Wilson method. No imputation of missing data was performed. Missing visits were excluded from the calculation.

Results of attack rate analysis

RSV-ARTI occurred in 15/149 two-person households (10.1% 95% CI 6.2-15.9%) and resulted in 18 RSV-ARTI cases [Table 3]. Three household partners became infected after being exposed to a primary infected household partner resulting in a secondary attack rate (SAR) of 21.4% (95% CI 7.6-47.6%). Influenza-ARTI occurred in 18/147 two-person households (12.2% 95% CI 7.9-18.5%) and resulted in 22 influenza-ARTI cases. Three household partners became infected after being exposed to an infected household partner resulting in a SAR of 17.6% (95% CI 6.2-41.0%). Although higher than the population risk (5.9% 95% CI 4.6–7.5%), the difference was not significant. The serial intervals for RSV were two, five and nine days (mean 5.3 days) while for influenza the serial intervals were two, three and four days (mean 3 days). Based on literature, 95% of influenza cases have an incubation period of less than 2.8 days [1], which would make one secondary case with a serial interval of two days uncertain. For RSV, 95% has an incubation period of less than 6.3 days [1] making two cases uncertain.

Average attributable fraction

Risk factor associations are often described using odds ratios or relative risks, which can be adjusted for confounders. These measures describe the amplification in disease risk in those with a particular risk factor compared to an individual without this risk factor. However, these metrics say little about the impact of the risk factor on the burden of disease over all cases, primarily because they do not take the prevalence of disease into account [2]. One can imagine that a moderate risk factor that is highly prevalent in the population still has a higher impact compared to a significant risk factor that is only present in a few people. The attributable fraction quantifies the proportional reduction in disease prevalence that would be achieved if the risk factor could be somehow eliminated from the population. To calculate the (unadjusted) attributable fraction (PAR) the following calculation is used:

P_e (RR-1) / 1+P_e (RR-1)

In which P_e is the proportion of cases being exposed to that risk factor and RR is the relative risk. While there are often multiple risk factors, we would like to correct the attributable fraction for correlations with other variables or confounders to prevent overestimating the effect of the tested risk factor. One can simply obtain the adjusted OR/RR from multivariable regression analysis and implement these estimates in the

attributable fraction calculation. An alternative approach involves first fitting a model for disease occurrence with all risk factors and confounders of interest. This model is used to predict the total number of cases that would have been observed in the dataset. By setting the risk factor of interest to zero (in a binary variable) while leaving the values of all other risk factors unchanged one can estimate the contribution of this variable to the model correcting for correlations and confounding. Sequential attributable fractions are obtained by removing multiple variables from this model and to estimate the proportional decrease in disease prevalence compared to the original prevalence. Therefore, the sum of the sequential contributions for each risk factor equals the combined attributable fraction for all risk factors in the model. The order in which risk factors are removed from the model is of importance for the estimation and is likely to yield different results depending on which variable is removed first. Average attributable fractions mitigate this problem by averaging the differing sequential attributable fractions derived from every possible permutation by which the risk factors could be eliminated from the model, while still keeping the nice property that the component attributable fractions sum to the combined attributable fraction. More detailed information about average attributable fractions and the Averisk package can be found in the paper by Ferguson and colleagues [2].

In our model, we tested ARTI as a function of child exposure (binary yes/no). The analysis was corrected for categorical variables: comorbidity, employment status, household size, having a living partner and daily contacts and continuous variables: age and Groningen Frailty Indicator Score.

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Table S1. Characteristics of study participants for the total cohort and household cohort

	Total study population N=1006	Household cohort N = 316
Demographics		
Age [median, IQR]	75 [69-80]	73 [68-78]
Female	534 (53%)	159 (50%)
Comorbidity (any)	680 (68%)	192 (61%)**
Cardiovascular	209 (21%)	58 (18%)
Pulmonary	116 (12%)	29 (9%)
Diabetes	78 (8%)	14 (4%)**
Frail[a]	142 (15%)[1]	25 (9%)***
Influenza vaccination	732 (77%)[2]	217 (71%)**
Pneumococcal vaccination	113 (12%)[3]	28 (10%)
Current smoker	79 (8%)	28 (9%)
Household smoke exposure	112 (11%)	45 (14%)*
Allergic (any)	261 (27%)[4]	88 (28%)
Hay fever	54 (6%)	14 (5%)
House-dust mite	31 (3%)	12 (4%)
Exposure		
Exposure to children < 5 years	502 (51%)[5]	166 (54%)
Never	493 (49%)	144 (46%)*
Infrequent (less than weekly)	310 (32%)	90 (29%)*
Frequent (weekly)	192 (19%)	76 (25%)*
Household composition		
Living alone	338 (34%)	0 (0%)
Living with others	668 (66%)	316 (100%)
Living with partner	641 (64%)	316 (100%)
Daily contacts [median, IQR]	5 [2-10]	6 [3-10]
Employed	120 (12%)[6]	41 (14%)

Abbreviations: ARTI = acute respiratory tract infection, IQR = interquartile range. Values are numbers and percentage of cases with that characteristic unless otherwise indicated. *p-value <0.05 **p-value <0.01 ***p-value <0.001 compared to those not in the household cohort. [a] Scored using the Groningen Frailty Indicator (GFI) questionnaire, a 15-item validated screening instrument to determine the level of frailty in adults, the cut-off for frail is at four points [3]. Missing data <1% is not shown, if more than 1% is missing, the percentages are added as footnote. [1]missing N=74 (7%), [2]missing N=52 (5%), [3]missing N= 89 (9%), [4]missing N=19 (2%), [5] missing N=11 (1%), [6] missing N = 38 (4%).

CHAPTER 7

WHO influenza-like-illness (ILI) underestimates the burden of respiratory syncytial virus (RSV) infection in community-dwelling older adults.

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Unpublished

ABSTRACT

Background

Respiratory syncytial virus (RSV) surveillance is heavily dependent on the influenza-like-illness (ILI) case definition from the World Health Organization (WHO) which is widely used for surveillance of acute respiratory tract infections (ARTI). It is uncertain whether ILI captures the burden of RSV in older adults. We aimed to assess the performance of ILI to detect RSV-ARTI in older adults.

Methods

The WHO ILI and the modified ILI (requiring only the feeling of being feverish) case definitions were evaluated in patients with PCR-confirmed RSV-ARTI from the community-dwelling older adults (≥ 60 years) that participated in the prospective European RESCEU cohort study.

Results

750 ARTI in 583 patients were analysed including 36 RSV-ARTI. Sensitivity for RSV was 33% for modified ILI and 11% for ILI due to a general lack of fever resulting in a three to 9-fold underestimation of RSV respectively. The AUC for both ILI case definitions to detect RSV was 0.52 indicating poor discriminative performance. Despite differences in symptomatology, no clinical symptom could exclusively distinguish RSV–ARTI from all other ARTI.

Conclusion

The incidence of RSV in community-dwelling older adults was underestimated nine-fold by using the ILI clinical case definition. Because worldwide RSV surveillance is largely dependent on this case definition, there is an urgent need to develop a better approach to measure future impact of introduction of RSV vaccines which are in late stages of clinical development.

INTRODUCTION

Acute respiratory tract infections (ARTI) are the leading cause of disease worldwide with an estimated incidence of 17.2 billion upper ARTI annually [1]. Lower respiratory tract infections are estimated to be responsible for 4.4% of all deaths worldwide in people of all ages, with higher rates in both ends of the age spectrum [2]. Amongst others, respiratory syncytial virus (RSV) is responsible for a significant part of this worldwide burden [3, 4]. Respiratory surveillance programs provide information for public health authorities used to minimize the impact of the disease by planning appropriate control and intervention measures and allocate health resources. The World Health Organization (WHO) uniformly collects worldwide data about ARTI epidemiology by using standardized case definitions [5, 6]. "Influenza-Like-Illness" (ILI) includes acute respiratory infection with fever (≥38°C) and cough and is commonly used as a case definition for respiratory infection. The performance to detect RSV in children and adults of case definitions that included fever such as ILI and severe acute respiratory infection (SARI) showed a wide range in sensitivity ranging from 24-86% [7-11]. However, older adult outpatients were not well represented in these studies. Increasing age can alter symptomatology which can result in a more atypical disease presentation in older adults compared to younger adults or children [12, 13]. Since RSV is known to cause an appreciable disease burden in the elderly population, we aimed to validate the performance of the WHO ILI case definition to capture RSV in a population of community-dwelling elderly adults.

METHODS

Study design

Participants from the RESCEU older adult study were studied. The design and data collection of the RESCEU older adult study has been described previously [14]. In summary, the RESCEU older adult study is a European multicentre, prospective, observational cohort study in community-dwelling adults of at least 60 years of age. Before the start of the 2017-2018 and 2018-2019 RSV season (1st of October – 1st of May), 1040 participants were recruited in 17 general practices in the Netherlands (Utrecht), Belgium (Antwerp) and the United Kingdom (Oxford). Participants with comorbidity were included as long as this was not live-threatening, caused immunodeficiency or would hinder in completing the study procedures. More detailed information about the RESCEU older adult study and the complete in- and exclusion criteria can be found at Clinicaltrials.gov, identifier: NCT03621930.

Study procedures

During the RSV season participants were followed up weekly by email or telephone by the local study teams. Home visits for viral testing were scheduled within 72 hours if one or more of the following ARTI symptoms were present for at least one day: nasal congestion or discharge, cough, wheezing or shortness of breath. Nasopharyngeal flocked swabs collected during the home visit were tested for RSV and influenza with a molecular point-of-care-test (POCT, Xpert® Xpress Flu/RSV assay, Cepheid, Sunnyvale, CA, USA) [15]. RSV-ARTI was validated after the study by in-house qPCR. During the home visit, vital signs including temperature, saturation, heart rate and respiratory rate were measured by the local study teams. All participants were instructed to complete a daily log to score the presence and severity of various symptoms for as long as symptoms were present for a maximum of 28 days [Supplemental File]. Participants had to indicate daily whether they measured their temperature or felt feverish, but they were not obliged to measure temperature daily. Participants from the RESCEU older adult study with an ARTI that was tested for RSV with POCT and/or qPCR and who completed a symptom diary during their illness were included in the current study.

Definitions

We validated the official ILI case definition from the WHO [5]. Because participants not always measured temperature, we also used a less stringent ILI-definition based on feeling 'feverish' ("modified ILI") [Box 1]. Fever was defined as a measured temperature

≥38°C at the moment of the home visit or as reported and measured by patients during the respiratory episode. Temperature was measured either auricular, axillary or orally. Feverish was defined as the feeling of being warm/feverish as reported daily in the diary. Symptoms were considered present if a symptom was reported for at least one day during the infectious episode. RSV-ARTI was defined as an ARTI with a positive RSV test result (POCT and/or qPCR positive for RSV). Those with a POCT confirmed influenza infection were classified as influenza-ARTI, while all other PCR negative ARTI were classified as 'other ARTI'.

Box 1. Influenza-Like-Illness (ILI) case definitions

ILI [5]	An acute respiratory infection with: - Measured fever ≥ 38 C° - And cough; - With onset within the last 10 days
Modified ILI	An acute respiratory infection with: - Feeling of being warm/feverish - And cough; - With onset within the last 10 days

Statistical analysis

Symptomatology was compared between those with RSV-ARTI, influenza-ARTI or other ARTI. We calculated the sensitivity, specificity, positive and negative predictive values of the ILI and modified ILI case definitions for RSV-ARTI. The discriminative accuracy of these case definitions for RSV-ARTI was assessed as a point on the Area Under the Receiver Operating Characteristic Curve (AUC). The case definitions were subsequently tested in a subcohort of participants with medically-attended (outpatient) RSV-ARTI (MA-RSV-ARTI).

Post-hoc analyses were performed to explore two alternative case definitions. First, differences in symptomatology between RSV-ARTI, influenza-ARTI, and other-ARTI were used to develop a case definition with a high sensitivity for RSV that could distinguish RSV from all other infections. The second aim was to make this first alternative case definition more specific for RSV by adding symptoms to furthermore distinguish RSV from influenza. Alternative case definitions had to be concise and easy to use in order to make them applicable for community surveillance. Similar to ILI, the performance of these alterative case definitions was tested in RSV-ARTI and MA-RSV-ARTI. Bootstrapping including 1000 random samples of 750 episodes with replacement was used for internal

validation of their performance in RSV-ARTI and MA-RSV-ARTI. No imputation of missing values was performed. All analyses were performed in R version 4.0.1.

RESULTS

Patients and infections

From the 1040 older adults that participated in the RESCEU study, 616 (59%) experienced at least one ARTI episode during study follow-up (range 1-5 ARTI). In total, 844 ARTI episodes occurred of which 805 (95%) were sampled during a home visit. Diary information was available in 750/805 (93%) ARTI episodes from 583 patients [Figure 1]. RSV was confirmed in 36/750 ARTI episodes while 57/750 ARTI episodes were influenza positive. The remaining 657 ARTI episodes were neither RSV nor influenza positive and thus classified as 'other ARTI'. Characteristics of the study population are described in Table 1.

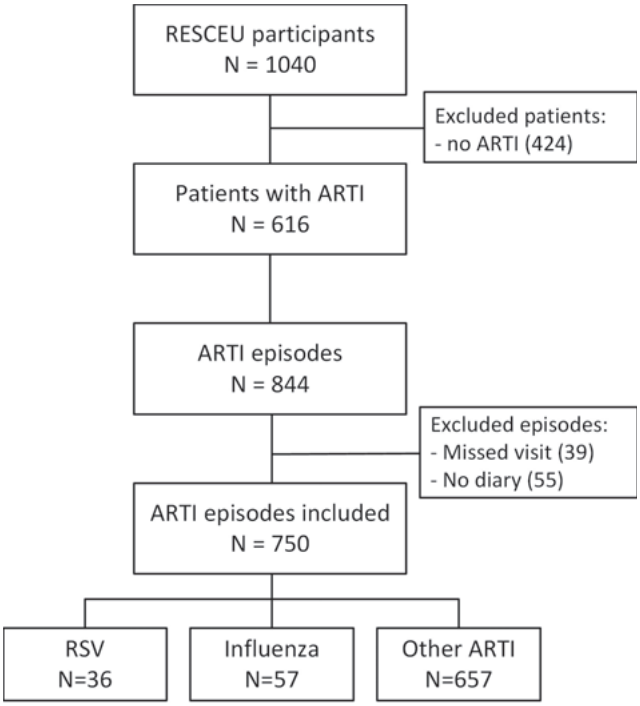

Figure 1. Flowchart of study participants and respiratory episodes.
Abbreviations: ARTI = Acute Respiratory Tract Infection.

Table 1. Characteristics of study participants

Characteristic	Patients with RSV N=36	Patients with influenza N=56 [a]	Patients with other ARTI N=583
Age:			
Median years [range]	75 [63-89]	71 [60-90]	75 [60-100]
Age above 75	20 (56%)	25 (44%)	302 (52%)
Female sex	20 (56%)	28 (49%)	320 (55%)
Comorbidity [b]			
Cardiovascular	7 (19%)	10 (18%)	121 (21%)
Lung	5 (14%)	7 (12%)	76 (13%)
Diabetes	2 (6%)	5 (9%)	59 (10%)
Allergies (any)[1]	11 (29%)	15 (27%)	167 (29%)
Hay fever	3 (9%)	2 (4%)	32 (6%)
House dust mite	0 (0%)	4 (7%)	22 (4%)
Pneumococcal vaccination[2]	4 (12%)	10 (21%)	71 (14%)
Influenza vaccination[3]	30 (86%)	44 (79%)	444 (79%)
Smoking status			
Current smoker	3 (8%)	5 (9%)	35 (6%)
Former smoker	14 (39%)	17 (30%)	241 (41%)

Numbers represent individual participants. Abbreviations: ARTI = acute respiratory tract infection. [a] One patient experienced two separate influenza B infections. [b] Cardiovascular comorbidity included all arrhythmias, structural heart diseases, angina and cardiac events such as infarction, percutaneous coronary intervention and bypass surgery. Hypertension was not included in this definition. Lung disease included asthma, COPD, chronic bronchitis and emphysema. Diabetes was defined as either type. Missing data <1% is not shown, if more than 1% is missing, the percentages are added as footnote. [1]missing N=12 (2%), [2]Missing N=56 (10%), [3]missing N=19 (3%)

Clinical symptoms

Patient reported symptoms and vital signs collected during the home visit are displayed in Table 2. Measured fever (≥38°C) was observed in 25% of influenza-ARTI, 11% of RSV-ARTI and 5% of other ARTI during the complete illness course. The feeling of being feverish was present in 65% of influenza-ARTI, 33% of RSV-ARTI and 29% of other ARTI. Patients with RSV-ARTI and influenza-ARTI more often experienced production of sputum (phlegm), dyspnoea and headache compared to other ARTI. Patients with RSV-ARTI and influenza-ARTI also more often felt ill and indicated more disturbances in their daily activities compared to other ARTI. Vital signs collected during the home visit showed that patients with influenza-ARTI more often had a fever compared to other ARTI (16% versus 2%, p=<0.001) but not compared to RSV-ARTI (6%, p=0.19). While an increased respiratory rate (>20/min) and lower saturation (<95% SaO2) were more often observed in those with RSV-ARTI and influenza-ARTI, these differences were not significant compared to other ARTI.

Table 2. Clinical symptoms of respiratory episodes

Patient reported symptoms	RSV-ARTI episodes N=36	Influenza-ARTI episodes N=57	Other ARTI Episodes N=657
Rhinitis	36 (100%)	55 (96%)	624 (95%)
Cough	35 (97%)	55 (96%)	572 (87%)
Wheeze	16 (44%)	26 (46%)	223 (34%)
Phlegm	34 (94%)	52 (91%)	466 (71%)**
Dyspnoea	24 (67%)	42 (74%)	309 (47%)*
Fever (≥38°C)	2 (6%)	11 (19%)	26 (4%)
Feeling feverish	12 (33%)	37 (65%)**	191 (29%)
Headache	27 (75%)	45 (79%)	348 (53%)*
Myalgia	19 (53%)	41 (72%)	263 (40%)
Disturbed sleep	26 (72%)	51 (89%)*	440 (67%)
Feeling unwell	33 (91%)	56 (98%)	499 (76%)*
Disturbance in daily activity	27 (75%)	51 (89%)	348 (53%)**
Vital signs from home visit [a]			
Temperature baseline (mean, SD)	36.4°C (0.5)	36.4°C (0.7)	36.5°C (0.6)
Temperature ARTI (mean, SD)	36.6°C (0.6)	36.9°C (1.0)*	36.5°C (0.6)
Temperature increase (mean, SD)[b]	0.2°C (0.6)	0.5°C (0.8)	0°C (0.7)
Fever (≥38°C)	2 (6%)	9 (16%)	13 (2%)
Respiratory rate (mean rate/min, SD)	17 (5)	17 (5)	17 (4)
Respiratory rate >20/min	6 (17%)	8 (14%)	63 (10%)
Saturation (mean%, SD)	96% (2)	96% (2)	97% (2)*
Saturation SaO_2 < 95%	5 (14%)	10 (18%)	39 (6%)
Heart rate (mean bpm, SD)	74 (12)	76 (12)	71 (11)
Composite fever (≥38°C)[c]	4 (11%)	14 (25%)	33 (5%)

Numbers represent respiratory episodes unless stated otherwise. Abbreviations: ARTI = acute respiratory tract infection; bpm = beats per minute. Statistical significance compared to RSV-ARTI is indicated by the asterisks: *P<0.05 **P<0.01 ***P<0.001 (not indicated if non-significant). [a] Measured by the study team during the ARTI visit unless otherwise indicated. [b] Compared to baseline measurement before the season [c] Patient reported or during ARTI home visit.

Performance of the ILI case definitions

Sensitivity of the ILI and modified ILI case definitions was low for RSV-ARTI (11% for ILI, 33% for modified ILI) [Table 3, Figure 2]. The AUC was 0.52 for both ILI case definitions. Performance of the case definitions in the subcohort of 178 medically-attended patients (11 medically attended RSV-ARTI) showed similar results [Table 3]. The proportion of RSV-ARTI within ILI was 7.5% while this proportion was 5.4% for modified ILI.

Table 3. Performance of ILI case definitions for RSV-ARTI

ILI [a]	RSV-ARTI N=36	MA-RSV-ARTI N=11
n(%)	4 (11%)	1 (9%)
Sensitivity (95% CI)	11 (3-26)	9 (0-41)
Specificity (95% CI)	93 (91-95)	84 (77-89)
PPV (95% CI)	8 (2-18)	4 (0-18)
NPV (95% CI)	95 (94-97)	93 (88-97)
AUC	0.52	0.46
Modified ILI [a]		
n(%)	12 (33%)	4 (36%)
Sensitivity (95% CI)	33 (19-51)	36 (11-69)
Specificity (95% CI)	70 (67-74)	58 (50-66)
PPV (95% CI)	5 (3-9)	5 (2-13)
NPV (95% CI)	95 (93-97)	93 (86-97)
AUC	0.52	0.47

Abbreviations: ARTI = acute respiratory tract infection; MA = Medically-attended; ILI = influenza-like-illness; PPV = positive predictive value; NPV = negative predictive value; AUC = Area Under the Receiver Operating Characteristic curve. [a] ILI includes measured temperature ≥38°C while the modified ILI includes also the feeling of being feverish.

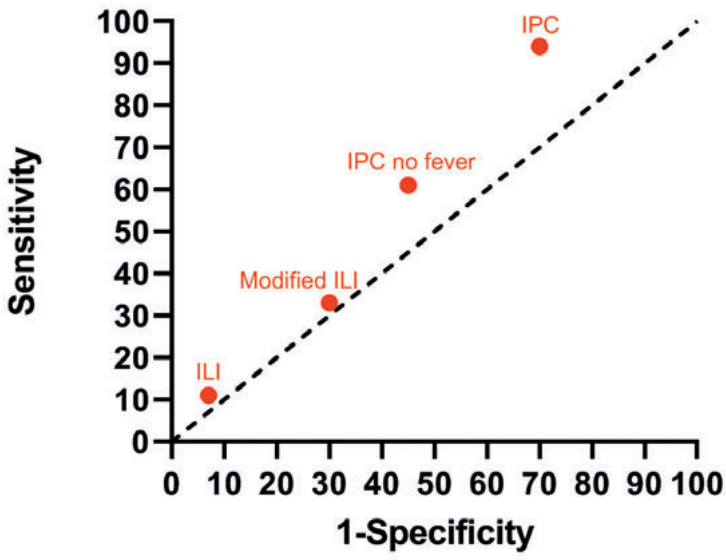

Figure 2. AUC plot of performance of case definitions for RSV.
Abbreviations: ILI = influenza-like-illness; IPC = infectious productive cough; ILI includes measured temperature ≥38°C while modified ILI includes also the feeling of being feverish.

Exploration of alternative case definitions

Infectious productive cough ("IPC") was defined as ARTI with cough and production of sputum/phlegm as a discriminating symptom to achieve high sensitivity to detect RSV-ARTI, but to decrease sensitivity for other infections. To increase specificity for RSV-ARTI and rule out influenza-ARTI we also defined IPC without fever or feeling feverish [Box 2].

Box 2. Alternative case definitions

Alternative 1: Infectious productive cough (IPC)	Acute respiratory infection with: - Cough with sputum/phlegm production
Alternative 2: Infectious productive cough (IPC) without fever	Acute respiratory infection with: - Cough with sputum/phlegm production WITHOUT - measured fever or feeling feverish.

Performance of the alternative case definitions

Infectious productive cough (IPC) captured 94% of RSV cases (2 missed cases) and 89% of influenza cases (6 missed cases), but specificity was only 30% [Supplemental Table S1]. This resulted in an AUC of 0.62 for IPC. The proportion of RSV within IPC was 6.4%. IPC without fever had a sensitivity of 61% for RSV while being less sensitive for influenza (sensitivity 32%). The specificity of IPC without fever for RSV was 55% resulting in an AUC of 0.58 [Supplemental Table S1, Figure 2]. The proportion of RSV within IPC without fever was 6.5%. Results in those with medical-attendance as well as results from internal validation using 1000 bootstrapped samples showed similar results [Supplemental Table S2-S3].

DISCUSSION

In this study we investigated the performance of the WHO ILI and a modified ILI case definition for capturing RSV infection in community-dwelling older adults. Sensitivity for RSV-ARTI was poor for ILI (11%) and modified ILI (33%) with an AUC of 0.52 indicating the inability of both case definitions to discriminate RSV-ARTI from other respiratory infections. Alternative case definitions formulated in this study were unable to substantially increase the performance to capture or distinguish RSV-ARTI. Complementary to previous studies performed in children and adults [7-10], we confirm that RSV is not captured well by the ILI case definition in community-dwelling older adults. Fever is less frequently reported in RSV infections compared to influenza infections [7, 16-19]. This was confirmed

in a recent study that validated the WHO case definition [7]. Age may influence occurrence of fever as shown for new-borns [8, 9, 20], but also for the elderly [21]. Elderly patients may lack a robust febrile response in up to one-third of acute infections, which can be caused by a lower baseline temperature (simply not reaching the 'fever' threshold) or because of diminished febrile responses [21]. As observed in our study, fever occurrence was low, which has a direct impact on the sensitivity of the ILI case definition for RSV even when the less stringent 'feeling feverish' criterion was used.

The strength of this study is the prospective design with a focus on the older adult community population. To our knowledge, this study represents the largest cohort of community-dwelling older adults to date in which the WHO ILI case definition is validated. Medical attendance was not required to trigger viral testing in those with respiratory symptoms which limits the risk of selection bias for more severe disease. Because of intensive follow-up, we tested 95% of the ARTI episodes and had complete symptom diary data available in 93% of those episodes.

Limitations also deserve discussion. First, the performance of case definitions is dependent on the clinical setting. Surveillance often takes place in medically-attended patients and not in community-dwelling patients although the latter used in this study provides an estimate for the optimal sensitivity of these case definitions when all symptoms experienced during an episode are considered. We observed mainly mild disease, although results were similar when applied to medically-attended patients only. Generalizability to hospitalized patients is uncertain since none of the RSV-ARTI episodes required hospitalization in our study. Second, patients were not obliged to measure temperature but could just have indicated feeling feverish what could have negatively affected ILI since ILI requires a measured fever to fulfil to the case definition. Temperature was measured in 97% of the home visits, but only 12% of RSV-ARTI visits exactly coincided with the peak of symptom severity. The median timing of the home visit in those with RSV-ARTI was one day after the peak of symptoms, while 71% were visited within two days. Additionally, temperature was measured orally, axillary and auricular. Oral and axillary temperature measurement might be less sensitive, measuring lower temperatures compared to rectal and tympanic membrane measurement [21]. However, 91% of patients were measured by the same method at baseline and moment of RSV-ARTI and temperature was not substantially higher upon respiratory infection. We used a modified ILI case definition requiring only the feeling of being feverish which was a

mandatory part of the symptom diary. Third, the number of RSV and influenza cases was low, which could have affected the performance of the case definitions. Bootstrapped results showed similar results and the observed symptomology was comparable to other studies [22, 23], which is reassuring.

Worldwide RSV surveillance is largely dependent on the ILI and ARI (Acute Respiratory Infection) case definitions [6]. ARI was not included in our analysis since we used ARI symptoms as criterion for sampling of ARTI episodes. ARI thus captured 99.6% of all ARTI in our study and was not specific for any pathogen. RSV vaccines for older adults are currently in late stage of clinical development. While clinical trials are able to show vaccine efficacy (performance of an intervention under ideal circumstances), we need good surveillance to measure vaccine effectiveness ('real-world' performance) after implementation. By using estimates of disease burden before and after implementation of a new vaccine in combination with vaccine coverage it is possible to calculate the impact of vaccine introduction. However, if the burden estimates are obtained from a surveillance system in which the majority of the burden is not captured, this will lead to an underestimation of the impact of vaccination. Because of the poor performance of ILI to capture RSV disease, we risk being unable to measure the impact of future RSV vaccine introduction.

Case definitions are used in surveillance for trend watching or to select patients for sampling in vaccine effectiveness (VE) studies. We show that the proportion of RSV in those who fulfil the ILI case definition is much lower for older adults (7.5%) [Table S4] compared to 19-45% for influenza [24, 25]. Consequently, even in the unrealistic scenario of a 100% effective vaccine, trends in case definition incidence would find at most a 7.5% reduction. These reductions might go unnoticed because of considerable variation in RSV seasonality [26] and suboptimal vaccination coverage and vaccination procedures in the 'real world'. Additionally, a reduction in RSV might be compensated by a higher occurrence of other respiratory pathogens. This phenomenon was seen for influenza were an unchanged incidence of ILI was observed despite a reduction in laboratory-confirmed influenza [24]. Despite our efforts to formulate alternative RSV case definitions, we were not able to increase the proportion of RSV and only showed a minor increase in discriminative performance [Table S1]. This suggests that there might not be a simple clinical case definition that can discriminate RSV from other respiratory

infections. Additional testing therefore seems inevitable to accurately determine the impact of RSV vaccination.

Vaccine effectiveness can be measured by determining the odds of vaccination between laboratory-confirmed cases and controls that fulfil a certain case definition using a test-negative design [27]. Larger studies will be required to accurately determine RSV vaccine effectiveness because of the lower proportion of RSV compared to influenza in currently available case definitions. To decrease the costs and limit the risk of selection bias in these studies the choice of which case definition to use for targeted sampling is crucial. The alternative case definitions from our study could improve this targeted sampling process. IPC enhances specificity compared to ARI while keeping a high sensitivity for RSV and influenza. Although ILI includes the highest proportion of RSV compared to the other case definitions, a large part of RSV cases will be missed, requiring more patients to be screened for ILI to find RSV cases [Table S4]. By using IPC the total number of patients that needs to be tested is diminished compared to ARI without the need of additional screening when ILI is used [Table S4]. The alternative case definitions are developed on a relatively low number of cases and validation in other settings and larger groups of patients is still needed to confirm their performance.

CONCLUSION

In this study we showed that the WHO case definition for ILI underestimated the RSV incidence by nine-fold in community-dwelling older adults. This is important since worldwide surveillance for RSV is largely dependent on the case definition for ILI. With the current surveillance programs, we risk being unable to measure the vaccine effectiveness of RSV vaccines. Since RSV vaccines are currently in late stage of clinical development, there is an urgent need to determine the best approach to measure the impact of RSV vaccine introduction in older adults.

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SUPPLEMENTAL FILE:

WHO influenza-like-illness (ILI) underestimates the burden of respiratory syncytial virus (RSV) infection in community-dwelling older adults.

Table S1. Performance of alternative case-definitions

IPC	RSV-ARTI N=36	Influenza-ARTI N=57	Other ARTI N=657
n(% of true cases)	34 (94%)	51 (89%)	447 (68%)
Sensitivity (95% CI)	94 (81-99)	89 (78-96)	68 (64-72)
Specificity (95% CI)	30 (27-34)	31 (27-34)	9 (4-16)
PPV (95% CI)	6 (4-9)	10 (7-12)	84 (81-87)
NPV (95% CI)	99 (97-100)	97 (94-99)	4 (2-7)
AUC	0.62	0.60	0.38
IPC no fever			
n(% of true cases)	22 (61%)	18 (32%)	301 (46%)
Sensitivity (95% CI)	61 (43-77)	32 (20-45)	46 (42-50)
Specificity (95% CI)	55 (52-59)	53 (50-57)	57 (47-67)
PPV (95% CI)	6 (4-10)	5 (3-8)	88 (84-91)
NPV (95% CI)	97 (94-98)	90 (87-93)	13 (10-17)
AUC	0.58	0.42	0.51

Abbreviations: ARTI = acute respiratory tract infection; IPC = infectious productive cough; PPV = positive predictive value; NPV = negative predictive value; AUC = Area Under the Receiver Operating Characteristic curve, values below 0.5 occurred since a prediction was made using a predictor (case definition) designed to detect another outcome (RSV) which led to a reversed prediction when tested in a different outcome than RSV.

Table S2. Performance of alternative case-definitions in medically-attended patients

IPC	RSV-ARTI N=11	Influenza-ARTI N=34	Other ARTI N=130
n(% of true cases)	11 (100%)	31 (91%)	105 (81%)
Sensitivity (95% CI)	100 (72-100)	91 (76-98)	81 (73-87)
Specificity (95% CI)	17 (12-24)	18 (12-25)	7 (1-18)
PPV (95% CI)	8 (4-13)	21 (15-29)	71 (63-79)
NPV (95% CI)	100 (88-100)	89 (72-98)	11 (2-28)
AUC	0.59	0.54	0.44
IPC no fever			
n(% of true cases)	7 (64%)	11 (32%)	66 (51%)
Sensitivity (95% CI)	64 (31-89)	32 (17-51)	51 (42-60)
Specificity (95% CI)	53 (45-61)	48 (40-57)	60 (44-74)
PPV (95% CI)	8 (3-16)	13 (7-22)	79 (68-87)
NPV (95% CI)	96 (89-99)	75 (65-83)	30 (21-40)
AUC	0.58	0.40	0.55

Abbreviations: ARTI = acute respiratory tract infection; IPC = infectious productive cough; PPV = positive predictive value; NPV = negative predictive value; AUC = Area Under the Receiver Operating Characteristic curve, values below 0.5 occurred since a prediction was made using a predictor (case definition) designed to detect another outcome (RSV) which led to a reversed prediction when tested in a different outcome than RSV.

Table S3. Bootstrapped performance of the alternative case definitions for RSV

IPC	Bootstrapped RSV-ARTI	Bootstrapped MA-RSV-ARTI
n(%)	NA	NA
Sensitivity (95% CI)	95 (82-99)	100 (70-100)
Specificity (95% CI)	30 (27-34)	17 (12-24)
PPV (95% CI)	6 (5-9)	8 (4-13)
NPV (95% CI)	99 (97-100)	100 (87-100)
AUC	0.62	0.59
IPC without fever		
n(%)	NA	NA
Sensitivity (95% CI)	61 (43-77)	64 (31-88)
Specificity (95% CI)	55 (52-59)	53 (45-60)
PPV (95% CI)	6 (4-10)	8 (3-16)
NPV (95% CI)	97 (94-98)	96 (89-99)
AUC	0.58	0.58

Abbreviations: ARTI = acute respiratory tract infection; MA = Medically-attended; IPC = infectious productive cough; PPV = positive predictive value; NPV = negative predictive value; AUC = Area Under the Receiver Operating Characteristic curve. Results of 1000 bootstrap samples of 750 cases with replacement out of the original data.

Practical application of the case definitions in RSV surveillance

We explored the performance of the case definitions in RSV surveillance. For the purpose of targeted sampling, we calculated the number of screened patients from all ARTI and the number of tested patients within a case definition required to identify an RSV case. ILI required the lowest number of tested patients per identified RSV case (13 cases) which was highest for ARI (21 cases). However, ILI required the highest number of screened ARTI patients per identified RSV case (188 cases), which was lowest for ARI (21 cases). IPC was efficient by requiring 23 screened cases to obtain 16 patients for diagnostic testing which was required to identify one RSV case [Table S4].

Table S4. RSV incidence within case definitions.

	Proportion case definition within ARTI	Proportion RSV within case definition	Total tested for RSV to find one case [a]	Total screened for case definition to find one case [b]
ARI	99.6%	4.8%	21	21
ILI	7.1%	7.5%	13	188
Modified ILI	29.7%	5.4%	19	62
IPC	70.9%	6.4%	16	23
IPC no fever	45.5%	6.5%	15	33

Abbreviations: ARTI = acute respiratory tract infection; ARI = Acute Respiratory infection; ILI = influenza-like-illness; IPC = infectious productive cough. [a] 1/ proportion RSV within case definition. [b] total screened = total tested / proportion case definition within ARTI.

Supplement: Diary Questionnaire

WEEK 1; symptoms each day

For each day, please give every symptom a score from 0 to 6. If you score 0 for all symptoms for two consecutive days, complete the weekly questions for week 1.

Score	Severity of symptom:
0	Normal/not affected
1	Very little problem
2	Slight problem
3	Moderately bad
4	Bad
5	Very bad
6	As bad as it could be

Symptoms	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
Cough							
Phlegm (coughing up slime)							
Shortness of breath							
Wheeze (during breathing out)							
Blocked/runny nose							
Muscle Ache							
Headache							
Disturbed sleep							
Feeling generally unwell							
Interference with normal activities/work							
Interference with social activities							

Please score your temperature daily;	Score	Severity of symptom
	.....°C	If measured, please write down the degrees Celsius
	0	Not measured but does not feel warm/feverish
	1	Not measured but does feel warm/ feverish

	Day 1	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
Temperature							

CHAPTER 8

Summary and general discussion

In this thesis I have addressed respiratory disease caused by, or related to, the respiratory syncytial virus (RSV) in two distinct populations; children and older adults. I will first summarize the main findings of this thesis after which I will discuss clinical implications and future perspectives.

MAIN FINDINGS

In part one of this thesis I discussed the prediction of severe RSV in the first year of life and its effect on development of long term respiratory sequelae up to six years of age. In addition, the agreement between different respiratory endpoints used to indicate asthma in children was investigated. In **Chapter 2** a Dutch prediction model was refined in the RISK study, a birth cohort study of 4088 late preterm born children recruited in more than 40 hospitals in the Netherlands. The final model included the following variables in order of importance: Day-care attendance and/or siblings, being born between 14th of August and 1st of December, neonatal respiratory support, breastfeeding ≤4 months or not, and maternal atopy. Discrimination of the model was acceptable with an AUC of 0.72. A nomogram was provided to calculate the individual hospitalization risk. The hospitalization risk can range from 0.4% (0 points) when no risk factor is present up to 20% (10 points) in case all risk factors are present. By applying a threshold of ≤4 points the risk of hospitalization is 1% which is comparable to a term born infant. By applying a threshold of ≥8 points, a high risk group is selected in which the hospitalization risk is 13% which is similar or higher to children in other high risk groups in whom palivizumab is used.

Participants of the RISK birth cohort study were thereafter followed up in **Chapter 3** at six years of age to investigate the association between early-life RSV hospitalization and ongoing symptoms of wheeze at school-age (six years old). Our study aimed to define how atopic predisposition changes the association between severe RSV infection and development of recurrent wheeze. An independent association was observed between RSV and current wheeze at age six. The association was only significant in children without atopy (aOR 4.05 95% CI 1.22-12.52), but not in those with atopy (aOR 1.50 95% CI 0.81-2.71).

It is challenging for physicians and researchers to make an asthma diagnosis. Numerous proxies of asthmatic disease have been used in literature and in clinical practice. **Chapter 4** describes our study in which the agreement was studied between common asthma

definitions (from herewith called "asthma indicators") in children from the WHISTLER birth cohort. The agreement between parental report, medical diagnosis and spirometry was also determined. Most agreement was observed between parents and doctors for objective, recent events such as a medical prescription while there was little agreement between lung function and clinical symptoms. While the prevalence of a history of symptoms was most consistent over time, they did not agree with the presence of current symptoms and showed significant recall bias over time based on parental report. These data advocate caution when literature with different asthma indicators is compared. Based on our results, I advise to use recent and objective indicators, such as a recent asthma diagnosis and the recent use of asthma medication because they displayed the highest agreement between parents and doctors with the lowest risk of recall bias over time.

In part two of this thesis I discussed the incidence, severity, symptoms and risk factors of RSV in the older adult community population. Results are based on the prospective international RESCEU older adult cohort study in 1040 community-dwelling older adults in Belgium, the United Kingdom and the Netherlands. In **Chapter 5** the burden of disease of RSV in these older adults is discussed. An overall RSV incidence of 5.7% (59/1040) was observed with an incidence of 4.2% in the first season and 7.2% in the second season based on both PCR and/or serology. Based on PCR-confirmed clinical ARTI, RSV had a lower incidence compared to influenza in the first season (1.9% versus 8.2%, respectively), but not in the second season (4.7% versus 3.3%). RSV infection had a longer duration of symptoms (19 versus 12 days, p=0.006) compared to other infections but not compared to influenza (19 versus 18 days). It was not possible to distinguish RSV from all other ARTI based on clinical symptoms. Medical attendance was sought in 31% of the PCR-confirmed RSV-ARTI, but no RSV-related hospitalizations or deaths occurred in older adults in this study. I therefore conclude that RSV is prevalent but rarely causes severe disease in this population of community-dwelling adults.

Young children are known to introduce RSV into households of young families, especially once they visit daycare or school. In **Chapter 6** I aimed to see how contact with young children under age five that did not live in the same household defined the risk of respiratory infection in older adults. Contact with young children living outside of the household increased the risk of all-cause respiratory infection and was highest in those with the most frequent contact (aOR 1.72 95% CI 1.18-2.49, p=0.005). This association

could not be confirmed for RSV specifically. By calculating the average attributable fraction of disease we determined that 10% of ARTI could be attributed to having contact with young children. Secondary attack rates within households in our study were calculated and showed that the risk of RSV-ARTI after being exposed to an infected household partner was 21.4% which was significantly higher compared to the population risk of 3.6% based on PCR-confirmed infection.

Surveillance conducted by public health institutes like the World Health Organization are likely to underestimate the RSV disease burden because surveillance is based on a system which was mainly developed to detect influenza. Case definitions, such as influenza-like-illness (ILI) are developed for influenza and may not fit well to the clinical syndrome of RSV-ARTI. How well RSV is captured in ILI in older adults was discussed in **Chapter 7**. RSV incidence was underestimated 9-fold when the WHO ILI case definition was used. This is important because while new RSV vaccines are being developed, surveillance might not be able to measure the impact of vaccine introduction because of how the system was designed. The performance of two alternative more specific RSV case definitions was investigated but failed to significantly improve RSV detection based on clinical symptomology. Additional testing therefore seems inevitable to determine the burden of RSV in the population. The alternative case definitions could play a role in more efficient targeted sampling for vaccine effectiveness studies.

GENERAL DISCUSSION

1. RSV IN CHILDHOOD

1.1 Prediction of severe RSV

Numerous studies have looked at risk factors of RSV hospitalization and at least five risk models have been published to predict severe RSV infection in early life [1-5]. Discriminative abilities of these models are good with AUCs ranging between 0.70-0.79. Some models have even been externally validated. Nevertheless, these models are not yet used in standard care. How can we explain this? Predictive models are used to select those with a high risk for intervention. Decreasing their risk can either be done by removing risk factors or by providing (preventive) therapy. For prevention of RSV only palivizumab is licensed which is costly. The American Academy of Pediatrics (AAP) recommends palivizumab for those born below 29 weeks gestation, those with significant heart disease, chronic lung disease of prematurity (CLD), and Down syndrome [6]. Most risk models have been developed for late preterm born children (32-35 wGA) [1, 2, 4, 5] or in term born infants [3]. None of the studied populations are therefore even considered to be high risk groups by the AAP unless they present with significant comorbidity. Nevertheless, our model described in **Chapter 2** distinguishes children with a hospitalization risk of up to 20% which is very comparable to the risk of high risk groups that are considered by the AAP.

Models could also be used to intervene on those factors that increase personal risk. If we look at the models in more detail we see that all include birth period and presence of older siblings. As described in the introduction, young age at the start of the season (<6 months) is a major risk factor because these infants experience the highest exposure during their most vulnerable period of life. Transmission studies have shown that older siblings are often the ones that introduce RSV in the household and are therefore a major source of infection for newborns [7-10]. These two factors contribute significantly to the performance of the models but provide little options for intervention except for good hand hygiene once siblings return from daycare/school. The parents could also decide not to admit their newborn to daycare to limit the risks although this is often not feasible. Other factors like atopic predisposition, birthweight and gender are inborn and cannot be intervened upon as well. It seems therefore that guided life style alterations are not a viable application as well. Both the high costs of palivizumab and the rigid basis of the most significant risk factors make it probable that none of these risk models are

currently being used in clinical practice. However, with new therapeutics on the horizon, there could be an important place reserved for risk models by selecting those who should receive these therapeutics.

1.1.2 Targeted prevention using risk models

Applicability of risk models depends much on the costs for the new therapeutic agent. When costs are similar to palivizumab (4717€ per infant per year [11]), targeted intervention in children with a >10% hospitalization risk using our prediction model from chapter 2 was not cost-effective compared to no intervention in cost-effectiveness analyses [11]. Threshold analyses in this study showed that when costs were lower (price below 2062€ per infant per year), targeted therapy would become cost-effective (below the 80.000€ per QALY threshold) [11]. This would allow for a more prominent role of risk prediction to identify those with a >10% hospitalization risk for preventive immunization. This analysis also showed that if pricing would be below 493€ per infant per year, the targeted intervention for high risk infants would actually be cost saving in this high risk population [11]. Palivizumab needs an average of five injections per child per winter to be protective, while the new extended half-life antibodies such as nirsevimab (MEDI8897, [12]) would only require one injection per season [13]. These new extended half-life antibodies are still in clinical development but we can speculate that prevention with such an agent (only requiring a single dosing) would be cost-effective below the 80.000€ per QALY threshold if it would be priced below 2062€ per infant per year and costs saving if it was priced below 493€ in high risk populations. Assuming that nirsevimab will be priced like vaccines such as the current pneumococcal vaccine (120$ per dose, requiring 3 doses = ~360$/320€ per child per treatment [14]), this would be below the cost saving threshold of 493€. Our prediction model could be used to identify high risk 32-35 wGA born children in whom preventive immunization using an extended half-life antibody is at least cost effective (assuming pricing will be below 2062€ per treatment). If pricing is much lower prevention might be costs effective in all premature infants. In that case, the role of risk prediction shifts to identifying high risk term born infants and requires development of a new prediction model that includes term born infants. The implications discussed in this section are derived from just one cost-effectiveness analysis and should be interpreted with care since some assumptions were uncertain and because there are multiple methods to model cost-effectiveness.

1.1.3 Gaps in knowledge of RSV prediction

There are gaps in knowledge about RSV prediction that still need to be investigated. As discussed, most models are being developed in (late) preterm infants and not in term born infants. Nevertheless, in absolute numbers most hospitalizations occur in those born term because there are simply more of them [15, 16]. To date, the only risk model for term born infants comes from the study by Houben and colleagues which did not predict hospitalization but LRTI in only 298 term born infants. We do not know whether we are able to select a high risk group (>10% hospitalization risk) among term born infants since their baseline risk is lower compared to preterm born infants. While it is safe to assume that many environmental risk factors will overlap, there are also distinct differences between these populations. Longitudinal birth cohort studies showed evident pulmonary consequences of being born prematurely that impact occurrence as well as severity of RSV infection [17]. This could explain the increased risk of RSV infection in those with neonatal respiratory support in our study which could reflect individuals with immature lung development and underlying impaired lung function. Post-hoc analysis in our study revealed a significant correlation between lower gestational age and higher occurrence of neonatal respiratory support strengthening this hypothesis. Ideally, a risk model should be developed based on a mixed population in which gestational age is also included in the model to correct for this fundamental difference. The ongoing European RESCEU infant cohort study will include 10.000 term born infants in a prospective birth cohort. Since the study design and questionnaires in RESCEU are harmonized with the RISK study, combining both studies will provide an opportunity to investigate risk factors in a prospectively followed mixed population.

Second, a prediction has to be made before the disease occurs. This requires identification of solid variables that are readily available at birth. Although we know that breastfeeding is protective, we have not yet identified a clear cut-off for how long it should be given. We used predicted breastfeeding in the RISK model but post-hoc analysis of actual breastfeeding showed that although parents can accurately predict whether they will breastfeed yes/no (>90% correctly estimated), they often overestimate how long they will breastfeed their child [Figure 1]. Neither "predicted breastfeeding, yes/no" nor any cut-offs of actual breastfeeding were significantly protective for RSV hospitalization in our study.

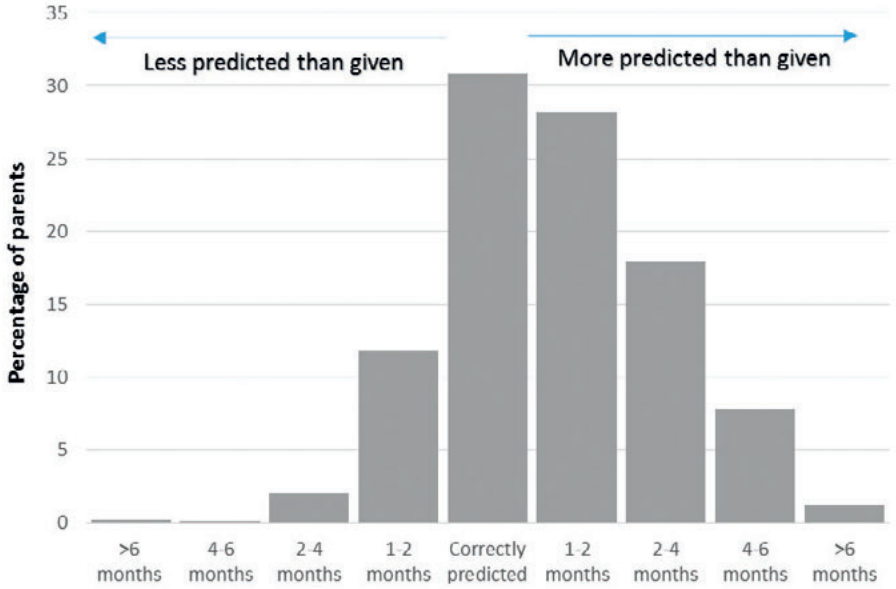

Figure 1. Actual and predicted duration of breastfeeding in RISK.

Parental atopy as a risk factor provides similar difficulties. Predictions at birth are dependent on the information available at that time which is the atopic status of the parents and not the child. The risk of atopic disease in the offspring is approximately 25% if one parent has atopic disease and 50% when both parents are affected [18]. The best predictive variable for atopic disease in the child is topic of debate. A recent systematic review found that parental history of atopic disease increased the risk of atopic dermatitis in the child independently of the affected parent's sex [19]. Furthermore, the association was strengthened when both parents were affected and when they had multiple atopic traits. The parental history of allergic dermatitis displayed a stronger association than parental asthma or allergic rhinitis did. To avoid data driven effects and to improve generalizability it would probably be best to use broad atopic markers such as atopy in neither, one or both of the parents.

Future models should include young age at the start of the RSV-season (<6 months), presence of siblings, planned day-care attendance and weeks gestational age (if a mixed term/preterm population or a wide range of prematurity is used). Other clinical factors may contribute to a small increase in performance of the model at the tradeoff of decreasing generalizability and ease of use. With new vaccines on the horizon, risk

stratification using prediction models may, or may not become more important which is largely dependent on the pricing of new therapeutics.

1.2 Does severe RSV infection predispose children for ongoing asthmatic symptoms?

The discussion about the association between RSV infection and asthma development is considered relevant according to the WHO but is still unresolved despite numerous observational studies, two randomized trials and meta-analysis [17, 20]. RSV is proposed to cause damage to the lungs resulting in sustained airway hyperreactivity while also affecting regulatory T-cell function leading to increased susceptibility to allergic airway disease [17]. Since atopy can predispose children to develop asthma as well as RSV infection (as discussed previously), it is hard to define whether RSV infection is merely a manifestation of a common predisposing factor or an individual risk factor for asthma development. Our paper discussed in **Chapter 3** was not meant to prove causality since we were well aware of the limitations of the study design. Our aim was to try to disentangle the effects of atopy and RSV infection on subsequent ongoing asthmatic symptoms. Stratification for atopic predisposition showed us that RSV infection did not significantly increase the risk of wheeze at age six in those with an atopic background. We observed that atopic children were already on a path towards asthmatic disease (indicated by the two-fold higher incidence of asthma in this group compared to non-atopic children). We hypothesize that RSV infection did not add much extra risk to this atopic group. However, we also observed that RSV was an independent risk factor in those without this atopic background. Although not emphasized in the MAKI trial (palivizumab – placebo controlled randomized trial [21]), similar effects of atopic status on the relation between RSV and parent-reported current asthma at age six were seen. Significant absolute and relative risk reductions between the treatment and placebo group were observed for current asthma in those without atopic parents, without allergic sensitization, without eczema and without allergic rhinitis but not in those with these atopic traits (appendix of the 2018 Lancet Infect Dis paper)[21]. Our study advises future longitudinal RSV studies (RCT and observational studies) to measure and stratify for atopic status. This is likely to be required in order to find an effect of (prevention of) RSV infection on ongoing asthmatic symptoms. Additional stratification for atopy will require an even larger sample size which will be costly and decrease feasibility. The sample size required to detect an effect of maternal vaccination for RSV on recurrent wheezing would already take tens of thousands of participants not even incorporating atopic stratification [22]. In contrast

to our study however, future studies may determine atopy directly in the participating child. This will provide a more accurate (and probably lower) estimate of atopy resulting in more power to detect a significant difference in the non-atopic group.

1.3 How should we define respiratory outcomes in RSV studies with long-term follow-up?

As discussed in the previous section, it will be challenging to perform one large study to determine the effect of RSV infection on persistent asthmatic symptoms. Alternatively, smaller RCT's could be bundled in meta-analysis to estimate an overall effect. To do this, we must make sure that outcome definitions are similar to prevent comparing apples and oranges. Unfortunately, standardization of outcome definitions is a big challenge in the field of pediatric asthma as observed in a systematic review of 122 published articles in which 60 different definitions of asthma or asthmatic disease were used [23]. The outcome of our study in **Chapter 3** was harmonized with the MAKI trial's outcome definition of parent-reported current asthma (current wheeze OR current use of asthma medication). However, based on reviewer's opinion we were pressed not to call this asthma but call it current wheeze. This is illustrative of the problem of what is considered asthma and what is not since two journals ended up with different names for a similar outcome (although the opposite is even worse). The MAKI trial showed a significant effect of palivizumab treatment for their primary outcome (parent-reported current asthma), but not for doctor's diagnosed asthma which led to the final conclusion that treatment did not have a major effect on current asthma. This shows that not only the definition, but also the source of information (i.e. parent or doctor) can result in a different conclusion. The most commonly used clinical definitions were compared in **Chapter 4** to determine the rate of agreement between definitions as well as between parents, doctors and spirometry. As suspected, considerable disagreement between definitions as well as between the sources that provided the information was observed. Most agreement between parents and doctor's was observed for objective recent events such as the recent use of asthma medication. We observed little agreement between historical outcomes ("Was your child ever diagnosed with asthma?") and presence of current symptoms. Considerable recall bias was observed in parental report of historic outcomes. The lack of agreement between spirometry and clinical symptoms was evident although we used simple spirometry without testing for reversibility. Current wheeze was often reported by parents but rarely recorded in medical registries after the first few years of life. This is of interest for our study in **Chapter 3** and the MAKI trial since both used parental-reported current wheeze

in their composite endpoint. Differences in current asthma between treatment groups in MAKI were predominantly caused by the report of infrequent wheeze by parents. Parental-reported wheeze could therefore explain the mismatch between the primary endpoint of parental-reported current asthma and doctor's diagnosed asthma. In **Chapter 4** I speculate that although wheeze can persist throughout childhood, parents and their children become familiar with this phenomenon and will no longer seek medical attention after initial experiences in early life. Although it is easy to conclude that these symptoms are therefore mild, there is evidence that they can still significantly influence quality of life [24]. I encourage future studies to use objective recent endpoints such as recent use of asthma medication or a current doctor's diagnosis of asthma. To asses a broader disease burden of respiratory sequelae one should acknowledge that medical registries will likely underestimate this burden.

2. RSV IN OLDER ADULTS

2.1 Burden of disease in RESCEU

Hospital-based studies have shown that RSV is a significant pathogen in adult patients who are admitted with respiratory infection [25-27]. These studies indicate that morbidity and mortality are similar to that of influenza and that the most severe disease occurs in those with older age and (cardiopulmonary) comorbidity [25-28]. Although the burden is well established in the hospital setting, much less is known about the impact of RSV in the community. Two earlier cohort studies have been performed over 15 years ago in community-dwelling older adults (those living independently at home) [25, 29]. However, both were monocenter studies and only the study by Ann Falsey and colleagues used PCR and serology to test for RSV while the study by Nicholson and colleagues only used serology. With our RESCEU older adult study we have performed the largest European study to date to provide sensitive, multi-country estimates about the burden of RSV in older adults in the community.

2.1.2 Incidence

RESCEU showed an RSV incidence of 4.2% and 7.2% confirming earlier estimates (3-7% yearly incidence [25, 29]) in this older adult population. Since influenza is a well-established problem for which vaccination is justified in this population, we established the significance of the incidence of RSV by comparing it to influenza. This was not straightforward in our study. We did not use serology to test for influenza so we were limited to the PCR-confirmed cases. Additionally, the 2017-2018 season was dominated by

a large influenza type B epidemic which contributed 66% of influenza cases that season and 47% of all influenza cases in our study. Based on the worldwide epidemiology of influenza, >50% dominance of influenza B over A occurs normally only once in seven influenza seasons [30]. Moreover, the total contribution of influenza B to all worldwide influenza is roughly 20%. We can therefore safely conclude that we experienced an extraordinary influenza season in our study. If we leave influenza B out of the picture and compare the incidence of RSV and influenza A in RESCEU we see that both are at least similar [Table 1], which was also concluded in the study by Ann Falsey [25].

Table 1. Incidence of PCR-confirmed RSV and influenza A in RESCEU and Falsey et al.[25]

Season	RSV	Influenza A
2017-2018 (RESCEU)	10/527 (1.9%)	14/527 (2.7%)
2018-2019 (RESCEU)	24/513 (4.7%)	17/513 (3.3%)
1999-2000 (Falsey)	12/212 (5.7%)	5/212 (2.4%)
2000-2001 (Falsey)	20/280 (7.1%)	5/280 (1.8%)
2001-2002 (Falsey)	5/180 (2.8%)	7/180 (3.9%)
2002-2003 (Falsey)	9/295 (3.1%)	7/295 (2.4%)

We should also consider those cases that were identified based on serology. In addition to the PCR-confirmed cases, 23 cases were only identified based on serology. Because serostatus flanking each ARTI was not determined, we can only speculate about the symptomatic fraction in those with positive serology. The majority (70%, 16/23 cases) of those showing seroconversion did experience at least one PCR negative respiratory infection during the months that RSV was circulating. While PCR can be falsely negative, it is unlikely that a four-fold increase in serology is false positive. Misclassification by PCR could have occurred, especially when testing was delayed. Seven cases showed seroconversion but did not report any respiratory infection during follow-up. These seven cases with isolated serology could have been truly asymptomatic although this is considered an exception for RSV which is almost always symptomatic [31, 32]. Unreported missed infection could also explain this inconsistency. Serology is an important addition to molecular testing (PCR) to capture the full burden of RSV-illness and most patients with serologic evidence of RSV experienced clinical respiratory infection during follow-up. Based on RESCEU I conclude that RSV is prevalent among community-dwelling older adults.

2.1.3 Clinical severity

Bronchiolitis in winter in a young baby has a high a priori chance of being RSV-related. Doctors even claim to be able to distinguish RSV based on a typical cough although a recent study showed that while sensitive, most doctors were not able to specifically identify RSV based on cough sounds [33]. With daily diaries, we obtained symptomatology from older adult cases in the RESCEU study. Although there were significant differences (especially between RSV and influenza versus other ARTI), no clear pattern of symptoms was identified that could validly distinguish RSV-ARTI from influenza and all other ARTI. Symptom duration was longest in those with RSV infection which was also most often unresolved after 28 days (22%). Persisting cough with sputum production was present in 6/8 (75%) of RSV-ARTI with symptoms beyond 28 days. Compared to PCR-confirmed RSV, influenza resulted in a higher proportion of medical attendance and antibiotic prescriptions (regardless of influenza A/B stratification). This may have been driven by the febrile syndrome which was observed in 65% of influenza-ARTI and only 33% of RSV-ARTI (as shown in **Chapters 5 and 7**). Patients that reported fever during their infection had a significantly higher proportion of medical visits (32% versus 20%, $p<0.001$) and antibiotic prescriptions (17% versus 5%, $p<0.001$) compared to those without fever. Although medical attendance and antibiotic prescription certainly influence costs, it does not necessarily have to indicate more severe disease. Neither RSV-ARTI nor influenza-ARTI showed increased frailty or worsening of pre-existing cardiopulmonary disease during follow-up. Symptom duration was comparable between ARTI caused by RSV and influenza. While influenza-ARTI was more severe than RSV-ARTI severe outcomes such as hospitalization and death were rare in our study. Roughly 75% of participants were vaccinated for influenza in our study. Based on the lack of severe disease we conclude that most RSV-ARTI were mild. Our findings suggest that watchful waiting, using a continuity of care approach to identify those who do need more intensive care is justified in case of suspected RSV infection in the community. Careful monitoring of patients with an increased risk of severe disease like those with cardiopulmonary comorbidity should be part of this approach.

2.2 RSV surveillance

Worldwide respiratory surveillance programs are increasingly used to estimate the incidence of RSV infection. However, these surveillance programs were designed for influenza surveillance. Case definitions such as Influenza-Like-Illness (ILI) are influenza specific and may therefore not be suitable to capture RSV. This shortcoming was

acknowledged which led to the use of the broader case definitions such as acute respiratory infection (ARI). We confirmed that by using ILI the incidence of RSV in older adults is underestimated by 9-fold (**Chapter 7**). We also show that ARI is very unspecific and resulting in a minor proportion of RSV within ARI that makes it inefficient for surveillance. Because there are no good RSV case definitions, the current respiratory surveillance programs will not accurately measure the impact of future RSV vaccines. Because RSV vaccines for older adults are currently in late stage of clinical development, there is an urgent need to determine the best approach to measure the impact of RSV vaccine introduction. For that purpose we explored alternative RSV case definitions that were formulated based on symptomatology observed in RESCEU. This showed to be difficult because respiratory symptoms were not very distinct from other respiratory infections. We could therefore not identify a clinical case definition that was able to specifically distinguish RSV-ARTI. Since the proportion of RSV within all existing and alternative case definitions was low (4.8-7.5%), even a 100% effective vaccine would still only result in a 4.8-7.5% decrease. This change may go unnoticed in light of the substantial seasonal variation and imperfect vaccination practices in the 'real world'. Additional viral testing seems therefore inevitable to determine the burden of RSV in the population. Targeted sampling based on case definitions could be used to decrease the costs of these surveillance programs or studies. Our alternative case definition of Infectious Productive Cough (IPC) includes cough with sputum production and captures almost all RSV and influenza cases while being more restrictive on the other infections. We show that by using IPC we can diminish the number of tests that need to be performed by 30% compared to ARI while still capturing >90% of RSV cases. The performance of this case definition should first be validated in a larger sample including more medically-attended patients.

Lessons learned and recommendations based on RESCEU:

1. RSV studies should be performed over multiple seasons because of seasonal variation.
2. Combined molecular testing and serology is required to assess RSV burden in older adults.
3. Nasopharyngeal samples should be collected within one week after disease onset to decrease the risk of misclassification.
4. Serum should be obtained before the season, and flanking acute infection to link serologic responses to clinical disease.
5. Good study adherence is achieved by investing in a thorough baseline visit during which all questions and expectations are discussed. Follow-up should be personalized in this age group, it is not a one-size-fits-all. Participants should be matched to the same study person if possible to increase adherence to the study.
6. Larger sample sizes or specific high risk subpopulations are needed to capture more severe outcomes such as hospitalization and death in older adult community populations.
7. Case definitions that include fever will significantly underestimate RSV incidence.

2.3 Should we vaccinate older adults for RSV?

Can we conclude that RSV is a burden in community-dwelling older adults? And if so, should we vaccinate older adults for RSV if such a vaccine becomes available? Based on the burden observed in hospitals the answer seems straightforward; high morbidity, ICU admission, high associated costs and mortality that is comparable to that associated with influenza [25, 26, 34]. This clearly paints a picture that would justify the need for protection. However, if we look at the general population, we see that RSV incidence is comparable to influenza with generally favorable outcomes and no increase in frailty. These reassuring population-based data do not support general vaccination against RSV of older adults.

2.3.2 High-risk groups

Which groups should be targeted if general vaccination of older adults is not applicable? There are groups at higher risk of severe infection. Prospective studies in patients with COPD and congestive heart disease (CHD) show that more severe disease occurs in these patients with hospitalization rates of 16-17% in those infected with RSV [25, 28]. These studies also show that RSV incidence is between 4-10% which is comparable to the general population and that despite their comorbidities 35% still experiences mild to asymptomatic disease [25, 28]. The increased risk of severe disease however would make them eligible candidates to receive preventive therapeutics. Another high risk group includes patients with immunodeficiency. Mortality rates of up to 55% have been reported in patients that are treated for (hematological) malignancies, hematological

stem cell transplants, or in those with solid organ transplants [35]. Protection of this very vulnerable group is justified, most likely using passive immunoprophylaxis before treatment onset or with new antiviral agents at the moment of acute illness. A third group includes those that live in long-term care facilities (LTCF). Based on a systematic review, incidence proportions ranged from 1.1 - 10.8% [36] although attack rates can be much higher upon an outbreak [37, 38]. These patients are again more prone to severe disease with hospitalization rates between 10-20% and mortality rates between 2-5% when infected with RSV [37].

2.3.3 Age, comorbidity and RSV

The independent role of older age in the occurrence and severity of RSV disease is widely accepted but is nevertheless unresolved. Immunosenescence occurring at older age may lower pre-existing antibody titers and make individuals more prone to infection. Impaired immune responses may result in more severe disease. However, in our RESCEU study we observe that antibody titers (neutralizing, pre-F and post-F) were not lower in those with older age but (cardiopulmonary) comorbidity was significantly more common in those with older age [Table 2].

Table 2. RSV Antibodies and comorbidity with increasing age in RESCEU

	Antibody titers median (range)			
Age category:	Neutralizing antibodies (µPRNT50)	Pre-F antibodies (EU/L)	Post-F antibodies (EU/L)	Cardiopulmonary comorbidity n(%)
60-69	595 (47-8157)	313 (39-2204)	227 (29-1638)	18% (54/303)
70-79	581 (53-18806)	357 (42-2547)	268 (22-2498)	30% (127/431)
80-89	607 (60-12143)	379 (58-5095)	328 (54-6395)	40% (111/276)
≥90	621 (124-1600)	524 (124-1600)	316 (119-1453)	60% (15/25)

Comorbidity may confound the relation between age and occurrence of RSV infection, especially when participants are recruited from medical settings because those with more severe disease are overrepresented. An increased incidence in those with older age was concluded in previous studies [39-41], often compared to those younger than 60 [40, 41]. None of the studies using multivariable corrected analyses concluded an independent effect of increasing older age above the age of 60 years old on either incidence or severity [40, 42-44]. We also did not observed a clear increasing incidence of RSV-ARTI with increasing age [Table 3]. Patients should be targeted for immunoprophylaxis based on underlying disease rather than age only. Target populations should include those

with COPD and (congestive) heart disease, severe immunodeficiency and those living in nursing homes.

Table 3. Incidence and severity of RSV stratified for age in RESCEU

Age category:	Incidence (PCR and serology)	Medical attendance (PCR only)	Symptom duration median days (IQR)
60-69	4.6% (14/303)	25% (2/8)	19.5 (16)
70-79	6.7% (29/433)	37% (7/19)	21 (13)
80-89	5.8% (16/278)	22% (2/9)	19 (18)
≥90	0% (0/26)	0%	NA

3. Vaccination strategies

3.1 Pediatric vaccination

Only palivizumab is currently available to prevent RSV disease which is by no means sufficient to diminish the worldwide burden given the many disadvantages. High costs, limited indications set by authorities, strict storage conditions, and monthly dosing schedules make widespread use not feasible, especially in low and middle income countries. Palivizumab is also not applicable for adults since it is dosed on body weight and is therefore too expensive in adults. Various new preventive strategies are investigated worldwide to provide more options to combat this virus. Immunization strategies should focus on protecting infants in the first six months of life since most severe morbidity and mortality occurs in this period [16, 45, 46]. An overview of possible preventive immunization strategies is shown in panel A of Figure 2. Active immunization can in theory be applied to all ages except for infants in their first months of life. These newborn infants may benefit less because of an immature immune system and presence of maternal antibodies that could interfere with response to vaccination [47]. Active maternal immunization during pregnancy could be an alternative strategy to protect these newborns in the first months of life. Maternal immunization may also provide indirect protection because the mother cannot transmit disease to the newborn after birth since she has been immunized [Figure 2, panel B]. Although protection during these first months of life is likely to have a major impact on the disease burden in this population [48], it is unlikely that this strategy can bridge the full extent of the critical first six months of life due to antibody decay [49, 50]. Additionally, it may not fully benefit those who are born prematurely since transplacental maternal antibody transfer increases during pregnancy and becomes most efficient during the later stages of the third trimester

[51]. If maternal vaccination fails (because of prematurity or underlying maternal disease) passive immunization right after birth can be an alternative to protect infants in the first months of life.

Passive immunization using newly developed extended half-life antibodies such as nirsevimab (MEDI8897) is assumed to be protective with only one dose per season, thereby decreasing costs and improving feasibility compared to palivizumab [12, 52]. These extended half-life antibodies can be given right after birth, providing protection for approximately the first five months of life, or can complement maternal vaccination when given around the third month of life. The advantage of passive immunization using a single dose given right after birth is that there is often contact with a health care provider and that the induced protection is likely to cover most of the vulnerable period of early life. While promising, much of the applicability of extended half-life antibodies depends on the pricing. Even with favorable pricing, maternal vaccines are likely to be cheaper which will ultimately determine the choice of preventive strategy in low income countries. In countries where there is clear RSV seasonality, it could be efficient to target mothers for maternal immunization who are likely to deliver just before or during the RSV season. These newborns will have an increased risk of severe infection as discussed before. We have evaluated whether seasonality should influence vaccination strategies in countries where there is a clear season from which we concluded that seasonal vaccination strategies for maternal vaccination is not feasible [Box 1]. Passive immunization could be efficient when given just before the season although this would require extensive logistics like for the seasonal adult influenza vaccination that may not outweigh the costs of just immunizing all infants at birth.

Since we know that siblings often introduce RSV in the household and are a major risk factor for RSV hospitalization in the newborn **(Chapter 2)**, pediatric vaccination of older children may block transmission of RSV to the newborn. This may delay the moment of primary infection and result in less severe disease [53]. To furthermore protect infants beyond the first months of life, either repeated passive immunization or active immunization can be considered. A combined strategy including maternal vaccination, passive immunoprophylaxis and active pediatric vaccination would clearly avert more RSV-related morbidity than any single intervention.

Box 1. Seasonal maternal immunization for RSV

"However, the advantages of seasonal maternal vaccination versus year-round maternal vaccination are not immediately evident. Currently, available maternal vaccines, including influenza subunit vaccines, provide protection for approximately two months after birth [50]. Timing is less of an issue since maternal vaccination before 30–32 weeks gestation results in a good antibody response [54]. Additionally, maternal vaccination will need to protect women with an expected date of delivery from two months before the start of the RSV season until well after the end of the season to also protect prematurely born children. Since this would imply maternal vaccination almost throughout the year, seasonal vaccination seems to offer little advantage over vaccination of all pregnant women."

Editorial Korsten et al. 2017 Lancet Public Health [55]

3.2 Older adult vaccination

The effects of preventive strategies in the older adult population are more difficult to predict. There are still some major hurdles to overcome in vaccine development. Immunosenescence makes it difficult to induce an effective immune response in older adults. Those that are most likely to benefit from vaccination are often also the ones least able to respond to vaccination [47]. It is also more difficult to assess the direct impact of vaccination on disease severity in older adults. Unlike children, the severity of the RSV infection in older adults may predominantly be the result of exacerbation of other preexisting comorbidities rather than the infection itself [47]. Studies should therefore evaluate a broader pallet of clinical outcomes to assess the full impact of preventive strategies. Another difficulty is the lack of immune correlates of protection from RSV for any age group. To identify to what extent the vaccine-induced immunity might achieve effectiveness, we need to assess the quality of the full vaccination response in the older adult population. Detailed comparison with humoral and cellular immune responses from younger healthy adults who did not develop severe disease is therefore crucial. Based on the results of an RSV adenoviral vector vaccine trial we have pointed out the importance of assessing and publishing both the cellular and humeral immune response since these data will benefit further vaccine development [Box 2].

Box 2. Evaluate a broad immune response in RSV vaccine trials.

"The key value of the study by Cicconi and colleagues is confirmation of the ability of viral vector vaccines to intrinsically promote a combined cellular and humoral immune response to one of the most important pathogens in infancy. In the absence of good correlates of protection against RSV, an open view in evaluating the broad immunoprotective effect of a new vaccine is justified."

Editorial Korsten et al. 2020 Clin Infect Dis. [56]

3.2.2 Alternative vaccination strategies

Are there alternative strategies to protect the elderly population? Older adults who are exposed to young children have a higher risk of respiratory infection (**chapter 6).** This was also observed for RSV specifically in patients with COPD [44]. Household studies have shown that RSV is often introduced in the household by school-aged/daycare visiting children [7-10]. Although we could not confirm this in our study, it would make sense that contact with children increases the risk of RSV infection in older adults outside of the household as well. Transmission is likely to occur by babysitting when there is both extended and physical contact between young children and their grandparents. Young children represent a significant viral reservoir for RSV and could therefore play a pivotal role in RSV disease dynamics and vaccination strategies. Vaccination of young children instead of babies and older adults has additional benefits over directly vaccinating these high risk groups. Young children have the advantage of a more mature immune system compared to babies and are likely to respond better to vaccination than older adults. Moreover, they lack the interfering maternal protective antibodies found in babies or RSV antibodies found in adults acquired from natural infections throughout life [47]. These pre-existing antibodies may interfere with the vaccine induced immune response in babies and make it harder to boost this pre-existing immunity in older adults. Another potential preventive strategy could therefore be to vaccinate these young children and thereby indirectly protecting both young babies and the elderly [Figure 2, panel B]. The scenario of vaccinating young children against RSV was modelled, and different mixing patterns with other groups were used to assess the indirect effects on other populations [53]. They observed that vaccinating young children aged 5-10 months was likely to protect infants <6 months of age effectively against hospitalization [53]. Unfortunately, they did not model the indirect effects of pediatric vaccination on the elderly population.

It is unknown whether herd immunity can be achieved for RSV, but by vaccinating those who spread most disease it may be possible to block transmission to those who are more vulnerable such as babies and the elderly. Much depends on a profound knowledge about mixing patterns used to determine who has contact with who and therefore who is likely to infect who. While mixing patterns in young families and between children have been studied and are reasonably well established, much less is known about contact patterns between children and older adults that do not live in the same household. Distinct differences in household composition around the world will play a role as well in the effectiveness of pediatric vaccination to protect the elderly. For example, the proportion of households that includes both a child under 15 years of age and an older person aged 60 years or over, is highest in Senegal (37%) and lowest in the Netherlands (0.2%) [57]. The overall benefit of pediatric vaccination may therefore actually be greater in non-western countries. We observed in RESCEU that frequent contact with children decreases with increasing age and frailty [Table 3]. One can imagine that those who are older and frailer have fewer social contacts and are therefore less exposed to RSV. While this might be true for western countries, the opposite can be true for developing countries where the frail elderly are cared for in the households of their children.

Table 3. Contact with children under five and increasing age and frailty in RESCEU

	Never	Infrequent	Frequent
Age 60-69 (N=301)	35%	32%	33%
Age 70-79 (N=426)	52%	33%	15%
Age 80-89 (N=275)	59%	30%	12%
Age ≥90 (N=26)	65%	31%	4%
Not frail (GFI<4, N=809)	47%	32%	21%
Frail (GFI ≥4, N=146)	56%	34%	10%

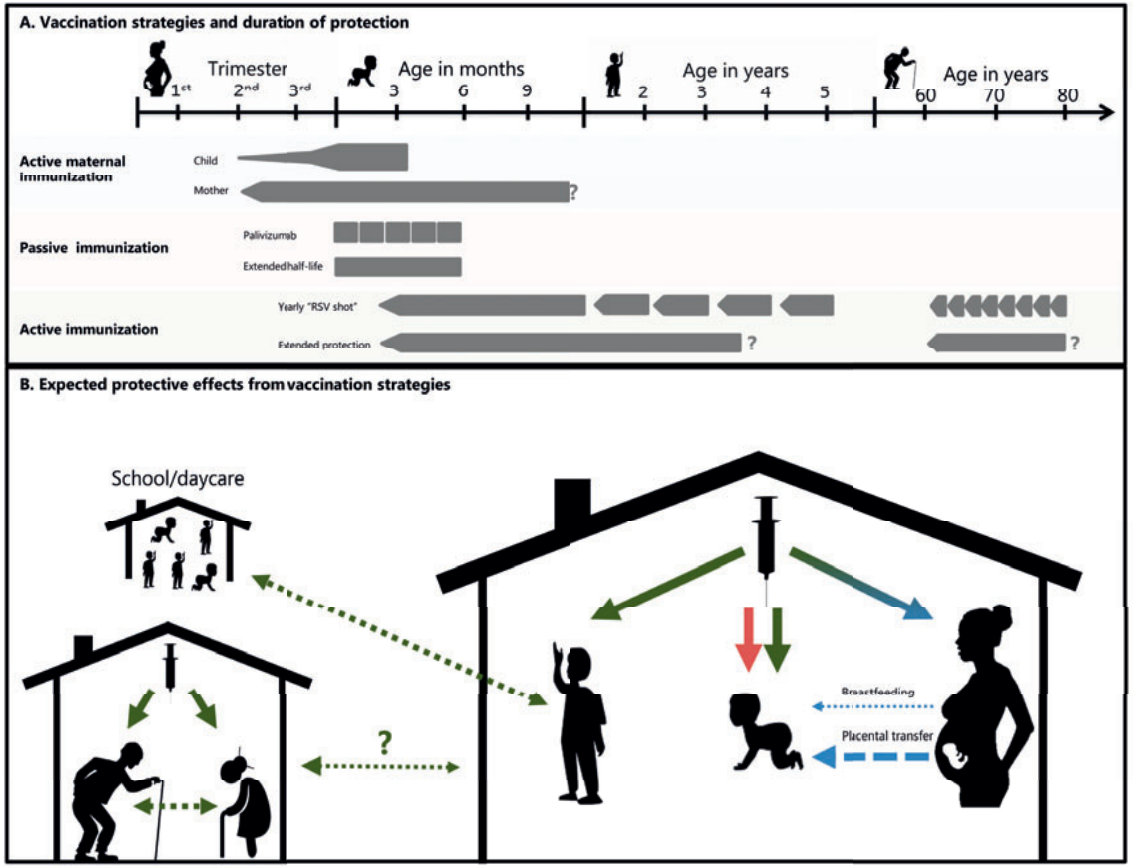

Figure 2. Vaccination strategies and their expected impact on RSV transmission in the household and community. Arrows represent the estimated protective effects of the different vaccination strategies. = maternal immunization during pregnancy. **Red** = passive immunization. **Green** = active immunization. **Blue** = maternal immunization during pregnancy. Full arrows represent direct vaccination effects, Dotted arrows represent indirect protective effects. The size of the arrow and the arrowhead matches the expected effect size of the intervention.

3.2.3 Lessons learned from influenza and pneumococcal vaccination

We can learn from the experience with mass vaccination of children for influenza and pneumococcal disease. Two large population-based studies have convincingly shown that the introduction of a conjugate vaccine against seven pneumococcal serotypes in the United States did not only result in a decrease in invasive pneumococcal disease (IPD) in those that were vaccinated, but also caused a significant decreased incidence of IPD in older adults [58, 59]. Similarly to the success of pneumococcal vaccination a national vaccination regimen of school-aged children in Japan against influenza has shown that this strategy also benefitted those not included in the program [60]. Initiated in the 1960's, this program was likely to be the cause of a significant decrease of excess mortality from pneumonia and influenza and even resulted in decreased all-cause mortality in all age groups after introduction, only to increase again after discontinuation of the vaccination program in the 1990's [60]. An extensive modelling study from the United Kingdom concluded that the most efficient way of reducing overall influenza-attributable morbidity and mortality appears to be to target the key spreaders, i.e. the children [61]. This modelling study and two other linked studies prompted a recommendation from the Joint Committee on Vaccination and Immunization to extend the influenza immunization program in the UK to all children aged 2–17 years in the summer of 2012 [62]. Cost-effectiveness studies have been performed since, which suggest that not only pediatric vaccination is highly costs effective, but that implementation has questioned the cost-effectiveness of immunizing older adults with low-risk [62, 63]. They do conclude that despite the effectiveness of pediatric vaccination, high-risk adult vaccination is still cost-effective [63]. The best evidence comes from a randomized trial were children aged 36 months – 15 years in Hutterite colonies in North-America were cluster-randomized to receive either influenza immunization or hepatitis A vaccination [64]. A significant protective effect of influenza vaccination was observed for community members in the intervention group [64]. These results suggest that pediatric vaccination against RSV may provide significant benefits for the protection of the older adult population as well, even in western countries. In my opinion the best way to protect the elderly population will include both active immunizations of high risk (older) adults complemented with pediatric vaccination in order to decrease the overall disease burden of RSV in the population.

CLOSING REMARKS AND FUTURE RESEARCH

In this thesis I have looked at disease caused by RSV in children and older adults. We should prioritize protecting newborn babies in their first months of life because of high morbidity and mortality (especially in low-middle income countries [45]). In addition, RSV also causes burden in older children and older adults. Epidemics of RSV infection put seasonal pressure on the healthcare system and have significant impact on those affected and their families. I have identified important risk factors for serious RSV disease in childhood and shown evidence that RSV infection contributes to ongoing respiratory symptoms in children that were not predisposed to develop these symptoms. In the second part of this thesis I have established the burden of RSV in the general population of older adults. I showed that RSV incidence is comparable to yearly influenza and that serious complications of RSV infection in older adults are rare. These results may advocate against widespread immunization against RSV when becoming available. However, cost-effectiveness studies are required to come to a more informed decision on RSV vaccination in older adults. We have shown that young children play a role in transmitting respiratory disease to the elderly population and therefore offer insight into novel preventive strategies. Last, I showed that RSV infection is not captured well in influenza surveillance systems indicating the need of better case definitions of RSV infection in older adults. Based on the gaps identified in this thesis I suggest the following for future research:

Future perspectives and recommendations:

1. To develop a prediction model for all newborn infants including term born infants. Risk stratification will be important because RSV vaccines will soon become available.
2. To define objective, recent outcome measures for asthmatic disease in RSV studies with long term follow-up and harmonize outcomes between studies to facilitate meta-analysis.
3. To perform large community-based studies to estimate severe complications such as RSV-related hospitalization in the general older adult population.
4. To perform household transmission studies at the homes of elderly to understand how exposure to infants increases the risk of severe RSV infection.
5. To update and validate alternative case definitions of RSV infection in older adults.

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CHAPTER 9

Nederlandse samenvatting

Het respiratoir syncytieel virus (RSV of RS-virus) is een veelvoorkomend virus dat opvallend genoeg relatief onbekend is in de samenleving. Elk kind krijgt RSV, u heeft het dus ook gehad. Voornamelijk bij jonge baby's in het eerste levensjaar veroorzaakt het RS-virus soms hevige luchtwegklachten waardoor alleen al in Nederland elke winter opnieuw 1500-2000 kinderen in het ziekenhuis moeten worden opgenomen. Nog jaren na de eerste infectie kunnen deze kinderen klachten van piepende ademhaling blijven houden. Na je eerste besmetting ben je er echter nog niet van af, je kunt namelijk de rest van je leven keer op keer opnieuw besmet raken met het RS-virus. De eerste infectie is echter vaak het ernstigst en opvolgende infecties geven veelal niet meer dan milde verkoudheidsklachten. Er zijn echter steeds meer aanwijzingen dat juist bij de oudere volwassenen, net als bij het influenza (griep) virus, er weer een hoger risico is op een ernstige infectie. In ziekenhuizen worden oudere volwassenen (en mensen met COPD, hartfalen en afweerstoornissen) opgenomen met ernstige longontstekingen die veroorzaakt worden door RSV.

Een behandeling voor RSV infecties is er niet en ernstig zieke kinderen worden ondersteunt met extra zuurstof, voeding en vocht tot ze zelf de infectie hebben overwonnen. Om RSV te voorkomen is er momenteel slechts één preventief middel geregistreerd tegen RSV; het humaan monoklonale antilichaam palivizumab, welke middels maandelijkse prikken tijdens de winter kan worden gegeven om kinderen te beschermen tegen ernstige RSV infectie. Palivizumab is echter erg duur (~5000€ per kind per winter) en wordt daarom slechts voor een beperkte groep hoog-risico kinderen vergoed. Dit zijn bijvoorbeeld kinderen die voor 32 weken zwangerschap worden geboren. Omdat er geen behandeling bestaat tegen RSV heeft het weinig toegevoegde waarde om voor RSV te testen omdat een testuitslag het klinisch beleid niet veranderd. Buiten het ziekenhuis, bijvoorbeeld in de huisartsenpraktijk, wordt er überhaupt weinig tot niet op RSV getest omdat het geen consequenties heeft en er nog veel onwetendheid is dat dit virus ook voorbij de kinderleeftijd luchtweginfecties kan veroorzaken. Dit heeft ervoor gezorgd dat we niet goed weten hoe vaak het RS-virus luchtweginfecties veroorzaakt bij oudere volwassenen buiten het ziekenhuis. Er wordt momenteel veel onderzoek gedaan naar medicijnen en vaccinaties die kunnen beschermen tegen RSV. Als deze vaccinaties beschikbaar komen (wat al op relatief korte termijn mogelijk is), moeten we weten wie er het meeste baat hebben om deze preventieve bescherming te krijgen. In dit proefschrift heb ik onderzoek gedaan naar (jonge) kinderen en oudere volwassenen om te kijken hoe vaak dit virus voorkomt en wie er het meeste risico loopt om een ernstige infectie te ontwikkelen.

Deel I RSV infectie op de kinderleeftijd

In het eerste deel van dit proefschrift heb ik gekeken naar ernstige RSV infecties op de kinderleeftijd en de gevolgen hiervan in de eerste levensjaren van deze kinderen. Dit heb ik gedaan aan de hand van de RISK studie, een geboortecohort van 4088 laat-prematuur geboren kinderen. In **Hoofdstuk 2** heb ik gekeken welke factoren een rol spelen op het risico voor deze kinderen om in het eerste levensjaar te worden opgenomen in het ziekenhuis met een RSV infectie. Hierbij zagen we dat kinderen met oudere broertjes/zusjes of die zelf naar de crèche/kinderdagverblijf gaan, kinderen die geboren zijn kort voor het RSV seizoen, respiratoire ondersteuning hebben gekregen na de geboorte, weinig borstvoeding hebben gekregen, of een atopische moeder hebben een hoger risico lopen op een ernstige RSV infectie. Met deze risicofactoren hebben we een voorspelmodel gemaakt om bij de geboorte eenvoudig het individuele risico op een ziekenhuisopname te berekenen. Dit risico loopt van 0.4% indien er geen risicofactoren aanwezig zijn (0 punten) tot 20% indien alle risicofactoren aanwezig zijn (10 punten). Door een afkapwaarde van ≤4 punten te gebruiken kan een groep kinderen worden geïdentificeerd die gemiddeld 1% risico heeft op een ziekenhuisopname. Dit risico is vergelijkbaar met gezonde, op tijd geboren kinderen. Andersom geeft een score van ≥8 punten een gemiddeld opnamerisico van 13% wat vergelijkbaar is met de hoog-risico kinderen waarvoor bescherming met palivizumab geïndiceerd is.

Zoals eerder besproken kunnen kinderen na een ernstige RSV infectie nog geruime tijd klachten houden van de luchtwegen. Meerdere studies hebben een verband gelegd tussen het doormaken van ernstige RSV infecties en het ontwikkelen van astmatische klachten al is de oorzakelijke rol van RSV in dit proces nooit bevestigd. Kinderen met een atopische aanleg (eczeem, hooikoorts, allergieën) hebben een hoger risico op het ontwikkelen van astma maar ook op het krijgen van een ernstige RSV infectie waardoor het moeilijk is om te bepalen of RSV de oorzaak is van de astmatische klachten, of dat de RSV infectie slechts een uiting is van de onderliggende gevoeligheid van de longen om hevig te reageren op prikkels zoals virussen en allergenen. In **Hoofdstuk 3** hebben we daarom bij een deel van de kinderen uit de RISK studie nogmaals een vragenlijst afgenomen op de leeftijd van zes jaar. Het doel was om te kijken of kinderen met een ernstige RSV infectie inderdaad vaker persisterende piepende ademhaling hadden, en of een atopische aanleg hier invloed op had. Een atopische aanleg was hierbij gedefinieerd als het hebben van op z'n minst één ouder met klachten van astma, hooikoorts of eczeem. In de gehele groep zagen we een verhoogd risico op een piepende ademhaling op de

leeftijd van zes jaar bij kinderen die in het eerste jaar waren opgenomen met een RSV infectie. Als we de gehele groep echter opsplitsten voor het wel of niet hebben van atopische aanleg, zagen we dat alleen bij de kinderen zonder atopische aanleg een RSV infectie het risico verhoogde op het ontwikkelen van persisterende piepende ademhaling op de kinderleeftijd. Wij adviseren dan ook om onderscheid te maken op basis van deze atopische aanleg in studies naar de lange termijn gevolgen van (behandeling voor) RSV infecties.

Een ander probleem bij het onderzoek naar astmatische klachten bij kinderen is dat de diagnose astma niet gemakkelijk te stellen is op de (jonge) kinderleeftijd. Dit gebeurt vaak op basis van waarschijnlijkheid en omvat een positieve klinische anamnese (piepende ademhaling, hoesten, kortademigheid), goede respons op een proefbehandeling met kortwerkende luchtwegverwijders of middels spirometrie waarbij kinderen voor en na het inhaleren van een luchtwegverwijder een longfunctie blazen om te kijken of dit de longfunctie verbeterd. Omdat kinderen pas rond hun zesde levensjaar voldoende in staat zijn om een betrouwbare longfunctie te blazen wordt de diagnose op de kinderleeftijd vaak gesteld op basis van symptomen en reactie op een proefbehandeling. Dit zorgt er echter voor dat in de klinische praktijk, maar zeker ook in wetenschappelijk onderzoek, grote verschillen bestaan in hoe astma wordt gedefinieerd. Een systematische review van de literatuur liet zien dat in 122 artikelen waarin astma de uitkomst was, er 60 verschillende definities van astma werden gebruikt. Het is echter onduidelijk of deze definities overeenkomen en met elkaar mogen worden vergeleken. Hiervoor hebben we in **Hoofdstuk 4** kinderen van het WHISTLER geboortecohort gevolgd op de leeftijd van vijf en acht jaar om te kijken hoe de verschillende astma definities zich tot elkaar verhielden. We vergeleken symptomen op basis van rapportage door ouders (vragenlijsten), dokters (huisartsenregistratie) en resultaten van de longfunctietest (spirometrie). We zagen grote verschillen waarbij de meeste overeenkomst te zien was als er recente en objectiveerbare klachten werden gebruikt voor de definitie. Hierbij kan bijvoorbeeld gedacht worden aan het recent gebruik van luchtwegmedicatie. Ook zagen we dat klachten die in het verleden plaatsvonden slecht overeenkwamen met de aanwezigheid van huidige klachten (“in het verleden behaalde resultaten geven geen garantie voor de toekomst”). Opvallend was dat longfunctie weinig overeenstemming liet zien met de aanwezigheid van luchtwegklachten. Wij adviseren dan ook om studies waarin verschillende astma definities worden gebruikt niet zomaar met elkaar te vergelijken. Voor nieuwe studies adviseren we het gebruik van

recente en objectiveerbare definities zoals medicatiegebruik of een recent door een dokter gestelde diagnose van astma.

Deel II RSV infectie bij oudere volwassenen

Studies vanuit het ziekenhuis naar oudere patiënten met luchtwegklachten laten zien dat het RS-virus ook in deze groep voorkomt en ernstige ziekte kan veroorzaken. Zoals gezegd in de inleiding wordt er, zeker bij volwassenen, weinig tot niet op RSV getest vanwege het gebrek aan klinische consequenties en onwetendheid over de rol van dit virus bij luchtweginfecties voorbij de kinderleeftijd. Er is daarom momenteel onvoldoende kennis over hoe vaak RSV bij oudere volwassenen in de samenleving voorkomt en hoe ernstig deze infecties verlopen. Om erachter te komen wat de omvang van dit probleem is hebben we de Europese RESCEU studie bij oudere volwassenen uitgevoerd. We hebben 1040 deelnemers in België, Nederland en het Verenigd Koninkrijk een winter gevolgd en elke keer als er luchtwegklachten waren hebben we getest op RSV. Deelnemers aan de studie waren ouder dan 60 jaar en woonden zelfstandig thuis. Gedurende twee seizoenen werden twee cohorten gerekruteerd en gevolgd waarin we in het eerste seizoen 22/517 (4.2%) patiënten en in het tweede seizoen 37/513 (7.2%) patiënten met RSV identificeerden. In **Hoofdstuk 5** worden deze patiënten beschreven en is te zien dat de incidentie van RSV vergelijkbaar was met dat van influenza A welke jaarlijks de griep veroorzaakt. Wel zagen we dat alle influenza (type A en B) vaker voorkwam dan RSV in het eerste seizoen door een grote uitbraak van influenza B in 2017-2018. Klachten veroorzaakt door RSV en influenza duurden gemiddeld respectievelijk 19 en 18 dagen wat langer was dan klachten bij luchtweginfecties die niet door een van deze virussen werd veroorzaakt (12 dagen). Patiënten met RSV bezochten in 32% van de gevallen een dokter voor hun klachten wat minder was dan patiënten met een influenza infectie (60%). Hoewel RSV regelmatig voorkwam veroorzaakte het geen longontstekingen, ziekenhuisopnames of sterfte en ging de kwetsbaarheid ("frailty") van deze patiënten er niet door achteruit. We concluderen dus dat RSV infecties met regelmaat voorkomen bij oudere volwassenen in de samenleving maar dat deze infecties slechts zelden ernstige klachten geven.

Omdat RSV in sommige groepen volwassenen wel vaker ernstige infecties kan veroorzaken worden er momenteel ook vaccins ontwikkelt om (oudere) volwassenen te beschermen tegen RSV infectie. Het is daarom belangrijk om te weten wie er een hoger risico lopen om besmet te worden. Omdat RSV veel voorkomt op de kinderleeftijd is het een logische gedachte dat volwassenen RSV krijgen via contact met (jonge) kinderen. Studies laten ook zien dat in jonge gezinnen RSV vaak in het huishouden wordt geïntroduceerd

door de kinderen die naar de crèche of naar school gaan. In **Hoofdstuk 6** hebben we gekeken of contact met jonge kinderen ook het risico op luchtweginfecties verhoogd bij ouderen die niet in hetzelfde huishouden wonen. We zagen dat contact met kinderen inderdaad een hoger risico geeft op luchtweginfecties en dat dit effect het sterkst is bij oudere volwassenen die wekelijks contact hadden met kinderen. Hoewel we dit effect ook verwachtten voor RSV, konden we dit in onze studie niet bevestigen. Op basis van het verhoogde risico op luchtweginfecties en het vóórkomen van contact met kinderen konden we berekenen dat 10% van de luchtweginfecties bij oudere volwassenen toe te schrijven was aan het contact met jonge kinderen. Dit gegeven kan belangrijk zijn bij het nemen van beschermende maatregelen maar kan ook invloed hebben op hoe er gevaccineerd moet worden. Het verminderen van contact met kinderen ten tijde van epidemieën, of het vaccineren van kinderen om zo de verspreiding van luchtweginfecties tegen te gaan kunnen zo mogelijk bijdragen aan een vermindering in het aantal infecties bij oudere volwassenen.

In het laatste hoofdstuk uit dit proefschrift hebben we gekeken naar surveillance programma's voor RSV. Gezondheidsorganisaties zoals het RIVM en de World Health Organization (WHO) maken gebruik van gestandaardiseerde 'case definities' die een syndroom van klachten omvatten om ziekte te kunnen meten. Dit is nuttig om op dezelfde manier het vóórkomen van ziekte op verschillende plekken en in de tijd te kunnen meten en vergelijken. Surveillance programma's zijn echter vaak opgezet voor het monitoren van influenza waarbij de 'influenza-like-illness' (ILI) case definitie wordt gebruikt. ILI omvat een acute luchtweginfectie met daarbij hoesten en koorts van ≥38 C°. In **Hoofdstuk 7** laten we echter zien dat koorts maar bij een klein deel van de patiënten met RSV voorkomt wat er toe leidt dat met het gebruik van ILI slechts 11% van de patiënten met RSV wordt geïdentificeerd. Dit geeft dus een forse onderschatting van de totale hoeveelheid RSV, zelfs als we gemeten koorts uit de ILI definitie vervingen door het gevoel koortsig te zijn (33% werd hiermee geïdentificeerd). Hierdoor lopen we het risico om het effect van introductie van een toekomstige vaccin te missen omdat we de daling simpelweg niet zouden detecteren met de huidige surveillance structuur. We hebben geprobeerd om een betere case definitie voor RSV te maken maar dat bleek niet mogelijk omdat RSV qua symptomen niet goed te onderscheiden was van zowel influenza als van andere luchtweginfecties. Wel kunnen deze alternatieve case definities bijdragen aan een effectievere manier van gericht testen op RSV wat nuttig kan zijn om de hoeveelheid deelnemers aan vaccinatie-effectiviteitsstudies te verminderen.

CHAPTER 10

List of publications

Dankwoord

Curriculum Vitae

LIST OF PUBLICATIONS

In this thesis:

Korsten K, Blanken MO, Nibbelke EE, Moons KG, Bont L; Dutch RSV Neonatal Network. Prediction model of RSV-hospitalization in late preterm infants: An update and validation study. Early Hum Dev. 2016 Apr;95:35-40

Korsten K, Blanken MO, Buiteman BJM, Nibbelke EE, Naaktgeboren CA, Bont LJ, Wildenbeest JG. RSV hospitalization in infancy increases the risk of current wheeze at age 6 in late preterm born children without atopic predisposition. Eur J Pediatr. 2019 Apr;178(4):455-462

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Korsten K, Adriaenssens N, Coenen S, Butler CC, Ravanfar B, Rutter H, Allen J, Falsey AR, Pirçon JY, Gruselle O, Pavot V, Vernhes C, Balla-Jhagjhoorsingh SS, Öner D, Ispas G, Aerssens J, Shinde V, Verheij T, Bont LJ, Wildenbeest JG on behalf of the RESCEU investigators. Burden of respiratory syncytial virus infection in community-dwelling older adults in Europe (RESCEU): an international prospective cohort study. Eur Respir J. 2020 Oct 15:2002688

Korsten K, Adriaenssens N, Coenen S, Butler CC, Pirçon JY, Verheij T, Bont LJ, Wildenbeest JG on behalf of the RESCEU investigators. Contact with young children increases the risk of respiratory infection in community-dwelling older adults in Europe – the RESCEU study. Unpublished

Korsten K, Adriaenssens N, Coenen S, Butler CC, Pirçon JY, Verheij T, Bont LJ, Wildenbeest JG on behalf of the RESCEU investigators. WHO Influenza-like-illness (ILI) severely underestimates the burden of respiratory syncytial virus (RSV) infection in community-dwelling older adults – a population-based study. to be submitted

Not in this thesis:

Korsten K, Bont L. Seasonal immunisation against respiratory syncytial virus disease. Lancet Public Health. 2017 Aug;2(8):e344-e345.

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DANKWOORD

En dan heb je opeens een boek geschreven. Een vreemde gewaarwording voor iemand die bekend staat om zijn algehele gebrek aan interesse voor boeken. Wat haalt het beste in je naar boven? Dat is je omgeving. Hoewel ik vaak te horen heb gekregen dat het promoveren me gemakkelijk af ging, denk ik dat dit alleen maar mogelijk is geweest door de geweldige groep mensen om mij heen. We hebben deze klus samen geklaard. "We" omvat veel mensen met wie ik in de afgelopen jaren heb gewerkt, maar ook mensen die juist helemaal niets met mijn onderzoek te maken hebben gehad en waar ik ook gewoon even Koos kon zijn. Zonder dalen geen pieken en geen 100% zonder soms ook eens lekker op 50% te mogen aanmodderen. Voor zover mijn levenslessen.

Gezien het dankwoord het meest wordt gelezen en ik het jullie niet kwalijk neem als je de rest van dit mooie proefschrift (nog?) niet hebt gelezen, hier nogmaals de belangrijkste boodschap: RSV veroorzaakt veel ellende op de kinderleeftijd maar stopt daarna niet! We moeten streven naar het indammen van dit virus bij zowel kinderen als oudere volwassenen om wereldwijd gezondheidswinst te kunnen realiseren. Ik hoop dat dit proefschrift bijdraagt aan de bewustwording van het probleem van RSV. Daarbij ontkom ik er niet aan om enkele voorvechters van deze missie te bedanken.

Allereerst wil ik alle deelnemers van de verschillende studies bedanken voor hun onzelfzuchtigheid en bereidheid om de diepe neuswatten te trotseren voor het groter goed, lang voordat deze door de COVID pandemie berucht werden. Zonder jullie geen onderzoek en zonder onderzoek geen kennis van zaken. Jullie deelname wordt ontzettend gewaardeerd!

Mijn promotor, Louis Bont. Louis, ruim een derde van mijn leven heb ik in jouw groep mogen werken. Ik heb je leren kennen als een ras-optimist wiens dromen en ambities velen, waaronder mijzelf, hebben geïnspireerd om het maximaal haalbare eruit te halen. Ik vind het bewonderingswaardig hoe jij zoveel verschillende projecten onder je hoede kunt hebben en nog steeds de cruciale details en pijnpunten van elke studie feilloos kunt identificeren. Jij bezit het vermogen om met een enkele aanpassing relevantie te geven en ziet altijd mogelijkheden waar anderen beren op de weg zien. Als mens ben je persoonlijk betrokken bij je medemens, ook op de momenten dat dit niet van je verwacht wordt. Niet elke baas komt je thuis bezoeken als je ziek bent, dat siert je.

Mijn copromotor, Joanne Wildenbeest. In één woord: 'wauw'! Wat kan jij een werk verzetten en toch zo bescheiden en vriendelijk blijven. Waar menigeen onder de enorme druk van zowel kliniek als onderzoek ergens te kort schiet, weet jij het niet alleen voor elkaar te krijgen om beide te combineren, maar ook nog om in beide te excelleren. Al kom je misschien niet zo over, je bent een echte power-vrouw. Ik wil van deze gelegenheid gebruik maken om te benadrukken hoezeer jij de drijvende kracht bent geweest achter het succes van RESCEU en daarmee mijn promotie.

De leden van de leescommissie; Prof. dr. M.H. Emmelot-Vonk (voorzitter), Prof. dr. M.J.M. Bonten, Prof. dr. P. Bindels, Prof. J.H.H. van de Wijgert, Prof. dr. Frenkel, en Prof. dr. T.J.M. Verheij, wil ik hartelijk bedanken voor hun tijd en bereidheid om mijn proefschrift te beoordelen in deze uitzonderlijke periode.

Loes en Brigitte, jullie zijn het kloppend hart van de RESCEU ouderenstudie. Jullie menselijkheid geeft studiedeelnemers het gevoel meer te zijn dan een studienummer. Dit is cruciaal voor de wederzijdse inzet en betrokkenheid bij de studie. Wat hebben we een hoop lol gehad met "onze oudjes". Van trombone-les tot peperkoekjes, van een boterham met potloodpunten (hagelslag) tot een pittoresk huisje in het bos, de anekdotes houden niet op. Ook de verdrietige zaken gingen we niet uit de weg. Van gestolen juwelen tot eenzaamheid, en samen naar de uitvaart van een van onze meest iconische deelnemers. Jullie geven het onderzoek haar gezicht en dat is een grote kracht. Hierin kunnen de werkstudenten ook niet ontbreken: Marin, Victor, Lieke, Lieke, Olivia en Merlijn, bedankt voor al het harde werk in de frontlinie met duizenden telefoontjes! Loes, ik ontkom er niet aan om jou in het bijzonder nog te noemen. Als mijn RSV-moeder was jij er vanaf het begin bij. Ik ken niemand die zo attent is als jij. Dat jij daarom aan mijn zijde wilt staan bij mijn verdediging geeft me ontzettend veel kracht.

Marieke, Kors en Christiana, dank voor jullie uitstekende begeleiding bij mijn uitstapje naar astma in de WHISTLER studie. Laat het Louis niet horen maar ik vond het heerlijk om ook even de oogkleppen af te doen en te beseffen dat er meer bestaat dan RSV.

Koffie, ooit had ik het streven om mijn koffieconsumptie gedurende mijn PhD te turven. Na een maand ben ik maar gestopt met tellen. Met wie kan je beter koffie drinken dan met je collega's en kamer/flexplekgenootjes. Even afreageren op die reviewer met dat onmogelijke commentaar, het vieren van successen met taart, of veelal gewoon kletsen over niets (of vrouwendingen, gezien de scheve man-vrouw verhouding). Alle Louis' PhD

Squad'ers, 1st floor party'ers en Epi[Epi$borrel==1,]'ers, dank dat jullie mijn liefde voor koffiepauzes deelden en weet; koffie = kwaliteit.

Bier, zonder ontspanning geen inspanning. U-town gang, Order van Hippocrates, Mannen van Utrecht, FC Flikkerpony's, Niek Achten (hoezo hebben wij geen groep?), bij jullie kan ik altijd aan de drukte ontvluchten om mijn hoofd leeg te maken. Of dat nou in de kroeg, op het voetbalveld, op vakantie, of met een mooi spel is. Dank!

Sieger, Griet, Anniek en Ferdy. Dank dat ik al ruim 10 jaar deel mag uitmaken van jullie familie! Mooie reizen hebben we gemaakt en vele feestdagen samen gevierd. Bij jullie ben ik altijd welkom en jullie oprechte interesse waardeer ik enorm!

Oma Miep, jouw arm staat op de voorkant van dit proefschrift en staat daarmee symbool voor een hele generatie "older adults". Jouw vitaliteit op de leeftijd van 92 jaar is bewonderenswaardig. Ik hoop dat ooit ook op die manier te kunnen bereiken.

Zonder fundering geen huis. Ab, Wies, Joep, Margo, zonder jullie geen Koos. Hoe meer we meemaken hoe sterker we worden.

Ab en Wies, jullie opvoeding heeft mij (en Joep) veel meegegeven waarvan ik nog steeds elke dag de vruchten pluk. Kwaliteiten als nieuwsgierigheid, oog voor je medemens, een gezonde dosis relativeringsvermogen en humor definiëren ons gezin. Soms onwetend werd ik hierin getraind. Geduld heb ik geleerd door een half uur op de bus naar het zwembad te wachten, omdat we die "per ongeluk" net hadden gemist. Jullie leerden mij om je over alledaagse zaken te verwonderen en vooral maar veel te proberen. Dit optimisme en de wijde blik op de wereld geven de rust en de mogelijkheid om je eigen pad in het leven te ontdekken. Zo'n opvoeding gun ik iedereen.

Joep, mijn broertje die inmiddels langer is. Ons leeftijdsverschil was vroeger nog wel eens een probleem. In de afgelopen jaren zijn we echter steeds meer naar elkaar toe gegroeid. Inmiddels ben je een volwaardig teamgenoot en ben ik vereerd dat jij mijn paranimf wilt zijn! Hoewel we veel eigenschappen delen geniet ik nog het meest van onze verschillen. Jouw bravoure en charisma werken aanstekelijk en openen vele deuren. De combinatie met een goed stel hersenen, die steeds meer aan het werk worden gezet, gaan je nog ver brengen. Aangezien jij wél een wetenschappelijke opleiding hebt gedaan twijfel ik daar niet aan!

Margo, door jou is dit dankwoord met een pagina verlengd. Ik was initieel wat kort van stof maar jij floot me hier terecht op terug. Misschien wist ik ook niet goed waar ik moest beginnen. Wat hebben we een hoop meegemaakt samen en wat ben ik blij dat jij al ruim tien jaar aan mijn zijde staat. Hoe gemakkelijk wij samen door het leven fietsen is bijzonder. Ruzie maken kunnen we niet, onze pogingen zijn lachwekkend. In mindere tijden sta je altijd voor me klaar. Samen gek doen maakt mij heel gelukkig en ik hoop dat nog lang samen te kunnen doen!

Bedankt!

CURRICULUM VITAE

Koos Korsten was born on the 7th of October 1989 in Utrecht, the Netherlands. After secondary school at the "Bonifatius College", from which he graduated in 2008, he studied medicine at the University of Utrecht. During his medical training he completed an internship abroad in Havana, Cuba where he studied the national preventive program against dengue fever in Cuba. In his last year he completed a senior internship at the pediatric ward in the Wilhelmina Children's hospital and at the emergency department at the Meander Medical Centre in Amersfoort. He took his first steps in medical research as a student-assistant when he started working for the RSV-research group in 2009. Initiated during a surgical internship in his third year of medical school, he performed a systematic review about the treatment of acromioclavicular dislocations which led to the publication of his first lead author paper. He expanded his interest in research during a scientific internship in the last year of medical training at the RSV-research group. He studied predicting RSV hospitalization in preterm infants from the RISK study for which he had been collecting data for over five years as a medical student. This article defined the start of this current thesis. After graduating from medical school in September 2015 he was offered the opportunity of starting a PhD in the RSV-research group under supervision of Prof. L.J. Bont and dr. J.G. Wildenbeest. In his PhD research he worked on multiple clinical studies including two studies from the European RESCEU project which investigated the burden of RSV infection in both children and older adults. He closely collaborated with various sites throughout Europe including Spain, Finland, Belgium and the United Kingdom. He combined his work as a PhD student with the postgraduate master Clinical Epidemiology at the University of Utrecht from which he graduated in July 2019.

In 2020 Koos started working at the pediatric ward as a resident not in training (ANIOS) in ziekenhuis Gelderse Vallei in Ede. Koos lives together with Margo in Utrecht ("me stadsie").