Asthma Exacerbations and Risk of Emergency Department Management Failure: Associations with Various Respiratory Pathogens in a Pediatric Population.

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List of abbreviations

ADV – adenovirus

AME – average marginal effect

AR - absolute risk

AUC – area under the curve

CI - confidence interval

CoV – coronavirus

DAG – directed acyclic graph

ED - emergency department

EV-D68 - enterovirus-D68

GINA - Global Initiative for Asthma

HC - health care

hMPV – human Metapneumovirus

HRV - rhinovirus

INF - influenza

ISAAC – International Study of Asthma and Allergies in Childhood

NAATs – nucleic acid amplification test

NIH - National Institutes of Health

OR - odds ratio

PASS - Pediatric Asthma Severity Score

PI - pulmonary index

PIV - parainfluenza virus

POC – point-of-care

PRAM - Pediatric Respiratory Asthma Measure

RCT - randomized controlled trials

RD - risk difference

ROC – receiver operating characteristic

RSV - respiratory syncytial virus

RT-PCR - real-time polymerase chain reaction

URTI – upper respiratory tract infection

Abstract

Asthma is the most frequent chronic illness of childhood. The highest burden of disease is defined by asthma exacerbations. In children, 60-80% of exacerbations are triggered by respiratory pathogens. The impact of respiratory pathogens on the exacerbation severity at presentation, on exacerbation evolution, and response to treatment, remains unclear.

First, I conducted a systematic review synthesizing the available evidence on the association between the presence of respiratory pathogens and clinical outcomes in children presenting with an asthma exacerbation. PubMed, EMBASE, BIOSIS and the Cochrane Central Register of Controlled Trials were searched up to October 2016. Title, abstract and full text of studies reporting on respiratory pathogen exposure and a clinical outcome: exacerbation severity, healthcare (HC) utilization, treatment response or morbidity, were screened in duplicate. The Risk of Bias In Non-Randomized Studies of Interventions tool was used for quality assessment. Twenty-eight observational studies (4224 children) reported on 112 different comparisons between exposure: any pathogen (n=45), rhinovirus (HRV; n=34), atypical bacteria (n=21), specific virus (n=11) or bacteria (n=1) and outcomes: exacerbation severity (n=26), HC utilization (n=38), response to treatment (n=19), and other indicators of morbidity (n=29). Using only comparisons with a mild to moderate risk of bias, we found a possible association between HRV and increased exacerbation severity on presentation (regression p-value 0.016) and between the presence of any pathogen and emergency department (ED) treatment failure (OR 1.57, 95% CI 1.04-2.37). The role of specific pathogens, co-infection, interaction with atopy, and the impact on other indicators of morbidity and HC utilization remains uncertain. The heterogeneity in the published data precluded data aggregation. The lack of good quality data led to limited strength of evidence.

Subsequently, I performed a secondary analysis and nested study of a large prospective cohort study, the DOORWAY-study, of 1012 children presenting to the ED with moderate or severe asthma exacerbation. First, we described the prevalence of respiratory viruses and specific bacteria present in children with an acute asthma exacerbation. Second, we investigated the association between the presence of any pathogen, HRV vs. non-HRV infections, specific viruses, and co-infection with the asthma exacerbation severity on presentation, defined as baseline Pediatric Respiratory Asthma Measure (PRAM) score. Finally, we determined the association between pathogen exposure and ED treatment failure (hospital admission, prolonged ED stay or return ED visit leading to prolonged ED stay or admission). Linear or logistic regression was used as appropriate, including interaction terms, to estimate absolute risks (AR) and risk differences (RD) with their 95%CI representing the average marginal effect across the total study population. We compared models using site as an independent covariate and a fixed effects 2-level hierarchical model.

Of the 958 respiratory specimens investigated, 591 (61.7%) were positive for one or more of the investigated pathogens, with HRV being the most single prevalent pathogen (29.4%). No association was found between any pathogen or co-infection with more than one pathogen and severity of exacerbation on presentation. Non-HRV pathogen presence compared to no pathogen, was associated with -12.9 percentage points (95% CI -19.5%; -6.3%) lower risk of severe exacerbation (AR 25.8% (95%CI 21.3%; 30.3%) vs. 38.6% (95%CI 34.1%; 43.2%), respectively). No pathogen specific positive association was found with severe exacerbation. HRV-A had the highest risk for severe exacerbation (AR 43.6%, 95%CI 34.7%; 52.6%); RD 5.7% (95%CI -4.5%; 15.8%)). The presence of human metapneumovirus (hMPV) and parainfluenza virus (PIV) was negatively associated with severity (RD -13.6% (95%CI -23.0%; -4.3%) and RD -31.7% (95%CI -44.5%; -18.9%)). No association was found between severity at

presentation and HRV-B, HRV-C, respiratory syncytial virus (RSV), influenza (INF), enterovirus serotype D68 (EV-D68), adenovirus (ADV) and coronavirus (CoV). The risk of treatment failure in the absence of a pathogen was 12.5% (95%CI 9.0%; 16.0%). The presence of any pathogen was associated with an 8.2 percentage points (95%CI 3.3%; 13.1%) increased absolute risk of treatment failure to an AR of 20.7% (95%CI 17.4%; 24.1%). There was an increased risk of treatment failure in children with a non-HRV infection vs. no pathogen: RD 13.1% (95%CI 6.4%; 19.8%). With regards to pathogen-specific associations, RSV, INF, and PIV were found to be associated with an increased risk (RD 8.8% (95%CI 0.4%; 17.2%), RD 24.9% (95%CI 4.7%; 45.1%); and RD 34.1% (95%CI 7.5%; 60.7%) respectively) of treatment failure. HRV species, hMPV, CoV, ADV, EV-D68 and co-infection were not associated with a statistically significant increased risk of treatment failure.

We conclude that there is insufficient evidence to suggest a positive association between specific respiratory pathogens and baseline severity of the asthma exacerbation. However, infections with non-HRV viruses as a group and more specifically RSV, INF and PIV were associated with increased treatment failure to bronchodilators and corticosteroids administered in the ED. Clarifying the role of a specific respiratory pathogen in children with asthma exacerbation will support the need for infection prevention and pathogen diagnosis on presentation and may open the way to future pathogen-adjusted treatment in children presenting with an asthma exacerbation.

Abrégé

1. L'asthme est la maladie chronique la plus fréquente chez l'enfant. La morbidité la plus importante est liée aux exacerbations de l'asthme. Chez les enfants, 60 à 80 % des exacerbations sont provoquées par des agents pathogènes respiratoires. Les conséquences des agents pathogènes respiratoires sur la sévérité de l'exacerbation au moment de la présentation, sur son évolution et sur la réponse au traitement, sont peu connues.
2. Une revue systématique synthétise les différentes conclusions rencontrées dans la littérature concernant l'association entre la présence d'agents pathogènes respiratoires et les résultats cliniques chez les enfants souffrant d'exacerbation de l'asthme. *PubMed, EMBASE, BIOSIS* et le *Cochrane Central Register of Controlled Trials* ont été examinés jusqu'à octobre 2016. Titres, résumés et textes d'études rapportant une exposition à un pathogène respiratoire et à un résultat clinique: sévérité de l'exacerbation, recours aux soins de santé, effet du traitement ou morbidité ont été révisés en double. L'outil *Risk of Bias In Non-Randomized Studies of Interventions* a été utilisé pour l'évaluation critique de la qualité de la méthodologie et le risque de biais. Vingt-huit études observationnelles (4224 enfants) ont rapporté 112 comparaisons différentes entre expositions [agent pathogène quelconque (n=45), rhinovirus (HRV; n=34), bactérie atypique (n=21), virus spécifique (n=11) ou bactérie (n=1)] et variables-réponse, soit la sévérité de l'exacerbation (n=26), le recours aux soins de santé (n=38), l'effet du traitement (n=19) et d'autres indicateurs de morbidité (n=29). En utilisant seulement les comparaisons avec un risque de biais léger à modéré, nous avons trouvé une association possible entre HRV et une sévérité accrue de l'exacerbation à la présentation (régression valeur-p 0,016) et entre la présence d'un agent pathogène quelconque et l'échec d'un traitement (RC 1,57; 95 % IC 1,04-2,37) aux urgences. Le rôle des agents pathogènes spécifiques, des co-infections,

d'une interaction avec atopie et les conséquences sur d'autres indicateurs de morbidité et le recours aux soins de santé demeurent mal définis. L'hétérogénéité des données publiées rend impossible l'agrégation des données. Le manque de données de bonne qualité limite la solidité des résultats.

3. Par la suite, une analyse secondaire et une sous-analyse d'une étude de cohorte prospective, la *DOORWAY-study*, a été conduite sur 1012 enfants qui se sont présentés aux urgences avec une exacerbation de l'asthme modérée à sévère. Tout d'abord, nous avons décrit la prévalence de virus et de bactéries présentes chez les enfants avec une exacerbation d'asthme aiguë. Puis, nous avons cherché une association entre un agent pathogène quelconque, HRV vs. la présence de pathogènes non-HRV, des virus spécifiques ou une co-infection, avec la sévérité de l'exacerbation de l'asthme à la présentation, définie par le *Pediatric Respiratory Asthma Measure* (PRAM), établie comme étant la mesure de base. Enfin, nous avons déterminé l'association entre l'exposition à un agent pathogène quelconque et l'échec du traitement aux urgences (admission à l'hôpital, séjour prolongé aux urgences ou retour aux urgences conduisant à un séjour prolongé aux urgences ou à une admission). Une régression linéaire ou logistique a été utilisée selon les cas, incluant les termes d'interaction pour évaluer les risques absolus (RA) et les différences de risque (DR) avec leur 95 % IC représentant l'effet marginal moyen pour toute la population à l'étude. Nous avons comparé les modèles en utilisant le site comme covariable indépendante et un modèle multiniveau à effets fixes (2 niveaux).
4. Sur les 958 spécimens respiratoires étudiés, 591 (61,7 %) sont positifs pour un ou deux pathogènes étudiés, HRV étant le pathogène le plus représenté (29,4 %). Aucune association n'a été trouvée entre un pathogène quelconque ou une co-infection avec plus

d’un pathogène et la sévérité de l’exacerbation lors de la présentation. La présence de pathogènes non-HRV comparée à l’absence de pathogène est associée avec un risque plus faible d’exacerbation sévère : RA 25,8 %; (95 % IC 21,3 %; 30,3 %) vs 38,6 % (95 % IC 34,1 %; 43,2 %) et DR -12,9% (95% CI -19,5%; -6,3%). Aucune association positive n’a été trouvée entre un pathogène spécifique et une exacerbation sévère. Le HRV-A représente le plus grand risque d’exacerbation sévère (RA 43,6 % (95 % IC 34,7 %; 52,6 %); DR 5,7 % (95 % IC -4,5 %; 15,8 %)). La présence du métapneumovirus humain (hMPV) et du virus parainfluenza (PIV) est négativement associée avec la sévérité (DR -13,6 % (95 % IC -3,0 %; -4,3 %) et DR -31,7 % (95 % IC -44,5 %; -18,9 %)). Aucune association n’a été trouvée entre la sévérité à la présentation et les HRV-B, HRV-C, le virus respiratoire syncytial (RSV), la grippe (INF), l’entérovirus de sérotype D68 (EV-D68), l’adénovirus (ADV) et le coronavirus (CoV). Le risque d’échec thérapeutique en l’absence d'agent pathogène est de 12,5 % (95 % IC 9,0 %; 16,0 %) dans la population à l’étude. La présence d’un agent pathogène quelconque est associée avec un risque accru d’échec de traitement : AR 20,7 % (95 % IC 17,4; 24,1 %); DR 8,2 % (95 % IC 3,3 %; 13,1 %), avec un risque accru d’échec de traitement chez les enfants avec une infection non HRV : RD 13,1 % (95 % IC 6,4 %; 19,8 %). Les recherches avec des pathogènes spécifiques, RSV, INF, et PIV sont associées avec des risques accrus (RD 8,8 % (95 % IC 0,4 %; 17,2 %); DR 24,9 % (95 % IC 4,7 %; 45,1 %) et DR 34,1 % (95 % IC 7,5 %; 60,7 %) respectivement) d’échec thérapeutique. Les espèces de HRV, hMPV, CoV, ADV, EV-D68 et co-infection ne sont pas associées avec un risque accru d’échec thérapeutique.

5. Nous concluons qu’une infection avec des virus non HRV en tant que groupe et plus particulièrement INF, RSV et PIV, est associée avec un échec de traitement au bronchodilatateur et corticostéroïdes administrés aux urgences. Nous n’avons pas

suffisamment de données probantes pour démontrer une association positive entre des agents pathogènes respiratoires spécifiques et la sévérité de l'exacerbation de l'asthme telle que mesurée à la présentation. La compréhension du rôle d'un agent pathogène respiratoire spécifique participe au besoin de prévention des infections, au diagnostic d'un agent pathogène à la présentation et à un traitement orienté en fonction de l'agent pathogène détecté pour les enfants se présentant avec une exacerbation de l'asthme.

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Preface & Contribution of Authors

As a first author of this thesis, I, *Joanna Merckx*, designed and drafted the initial thesis and approved the final version for submission. As first author of the systematic review, I designed and executed all phases of the review and wrote the initial manuscript. As the first author of the second manuscript, I designed the analysis methodology, performed the analysis and drafted the initial manuscript. As a thesis supervisor *Caroline Quach* oversaw and directed the thesis question, methodology, revised and approved the final version of the thesis. For the first and the second manuscript, she supervised the study design and first author, provided guidance on analysis interpretation and critically reviewed the manuscript. As thesis co-supervisor *Francine Ducharme* critically reviewed the thesis and approved the final version of the thesis. She critically assessed the manuscript of the systematic review. For the second manuscript, she provided the data, provided guidance on the study design and interpretation of results and critically assessed the manuscript. As thesis committee member, *Christine Martineau* supervised the laboratory analysis of the sub-study of the data used in the second manuscript, provided guidance on interpretation on laboratory results and critically reviewed the manuscript. For the first manuscript, *Hannah Kraicer-Melamed*, as a co-author, assisted in records and full text screening, quality control of the data extraction and critically reviewed the final manuscript. *Genevieve Gore*, as a co-author, helped conceptualizing the search strategy and reviewed the manuscript. For the second manuscript, the secondary analysis of the DOORWAY study, the original study co-authors agreed in use of the data. All authors and co-authors of the 2 manuscripts, approved the manuscripts for use in this thesis.

Chapter 1: Introduction

I. Asthma and asthmatics

Asthma is the most frequent chronic diseases of childhood (1, 2). Asthma is a chronic disease of the airways characterized by difficulty of breathing, wheezing and cough. Airway inflammation with airway hyper-responsiveness and bronchoconstriction, leading to narrowing of the airways, are the main components of asthma pathophysiology. The adaptive immune system, with prominence of T-helper type 2 (TH2) cells, along with the innate immune system (3) are modified in children with asthma and orchestrate the exaggerated inflammation of the airways present in asthma and asthma exacerbations.

Asthma is a chronic disease of both childhood and adulthood, with onset usually at a young age. It is a heterogeneous disease with different phenotypes, classified as persistent or intermittent asthma, based on the temporality of symptoms (4, 5). Its evolution and trajectories over a patient's lifetime are diverse, with a group of children outgrowing their asthma and those with more severe and persistent asthma at a young age having a higher risk of remaining asthmatic (6).

Asthma can be one of the presenting diseases of atopy. Atopy is defined as the tendency to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens (7) and is documented by the presence of IgE antibodies in serum or by a positive skin prick test (8). Allergy and allergic mechanisms play a role in about half of the children with asthma.

Asthma diagnosis is based on clinical symptoms and confirmed by the use of lung function tests, documenting airflow obstruction and reversibility to bronchodilator or inhaled therapy (9, 10). The use of lung function tests has been historically limited to children above the age of 6 years, because of the decreased capacity to perform acceptable and repeatable spirometry curves in younger children. Although more recent work has proven that lung function testing is possible in

the clinical setting from the age of 3 years old, the diagnosis in younger children remains based primarily on the clinical demonstration of airflow obstruction and reversibility, the absence of alternative diagnosis, and at least two episodes of wheezing (4, 5, 9, 10). Asthma diagnosis can thus be challenging. Most guidelines suggest that children with 2 or more wheezing episodes are at high risk of having asthma and are considered as asthmatics. This is based on the criterion validity in predicting the presence of asthma (11), which has been proposed by the Canadian Thoracic and Paediatric Society (12), and used in published peer-reviewed literature (13, 14). GINA-, NIH-guidelines and country-specific guidelines have tried to uniform the diagnosis and the treatment of asthma. Asthma therapy is primarily based on the use of controller therapy, usually inhaled corticosteroids, and rescue treatment with bronchodilators (short acting beta-2-agonists). Treatment aims are to achieve and maintain long-term asthma control and prevent future risks, including exacerbations and impaired lung growth and function. Up until now, there has not been any curative treatment for asthma.

II. Asthma Exacerbations

An asthma exacerbation is a temporary worsening of asthma symptoms, characterized by bronchoconstriction, airway inflammation and mucus secretions, that is troublesome for the patient and prompts the need for a treatment intensification (15). Common triggers of exacerbations are exposure to allergens, tobacco smoke, temperature and humidity changes, exercise, stress or respiratory pathogens (16). The definitions used to define an asthma exacerbation differ widely in the literature. Efforts have been made to harmonize diagnosis and to enable comparisons in asthma research (17). The most widely used definitions include the need for intensified treatment and/or an unscheduled asthma related health care visit. Exacerbations are

thus often diagnosed based on the use/intensification of medication, namely of short and long acting beta-agonists and/or systemic corticosteroids. They can also be clinically diagnosed by a physician by the use of symptom scores which is based on clinical change in symptoms compared to the patient baseline or by documentation of respiratory signs and symptoms. Asthma exacerbations are classified by their severity and often categorized as mild, moderate or severe, using GINA or NIH guidelines (9, 10). Different validated instruments are used for severity classification on presentation, including Pediatric Asthma Severity Score (PASS), Pulmonary Score, Pediatric Respiratory Asthma Measure (PRAM), Clinical Asthma Score , Pulmonary Index (PI) and Asthma Severity Score (17). Exacerbations can be mild but also be life-threatening and differs between and within patients. Asthma morbidity and costs are mainly due to exacerbations and associated hospital admissions (18). They also contribute to anxiety in patients and their parents. Exacerbations are indicators of poor asthma control and are largely preventable events through better diagnosis of asthma in children, improved (maintenance) treatment of the chronic condition and, where possible, trigger avoidance. Asthma exacerbations are treated depending on their severity on presentation. Mild exacerbations are treated with intensification of short-acting bronchodilator use. Moderate or severe exacerbations are treated with short courses of systemic corticosteroids and severity-specific bronchodilator treatment. Symptomatic treatment with oxygen and respiratory support are needed in more severe cases, which may include the need for hospitalization, and occasionally admission to the intensive care unit.

III. Asthma Epidemiology

It is estimated that over 300 million people suffer from asthma worldwide (19). Its burden, assessed by disability-adjusted life years, puts asthma in the top 20 conditions affecting people of

all ages (1, 20). Our knowledge on the prevalence of asthma improved through International Study of Asthma and Allergies in Childhood (ISAAC) standardized collected data, involving 306 centers in 105 countries (21). Standardized questionnaires are the gold standard in the assessment of asthma prevalence in populations.

There are large variations in the prevalence of asthma in children of all countries, with a trend to greater asthma prevalence in Western countries. Countries in the developed world have a prevalence of asthma of > 10% in children 8-to-9 year old. Australia has the highest prevalence (31% in 2002 in 8-11 year-olds) (22). Asthma affects more than 1/2 million Canadian children aged 4 to 11 years (2), with the greatest burden found in preschool children. Prevalence data are however scarce from most developing countries, with very limited data from the African continent. Initially it was believed that asthma wasn't an important disease in developing countries, however recent data, where available, show an increasing prevalence. Prior under-recognition of the diagnosis of asthma might be underlying this lag in prevalence data. Also misclassification of asthma as pneumonia keeps underestimating the burden of childhood asthma in developing countries. Low- and middle-income countries, however, contribute to the highest number of asthma-related deaths (23).

Asthma and atopy related diseases (allergy and eczema) have increased in prevalence over the last 50 years (22), which has been ascribed to changes in environmental factors. Direct association between air pollution and increased inception of asthma has not been shown, however other environmental factors like high intensity truck traffic exposure, tobacco smoke exposure, open fire cooking, dampness and molds in homes and others have been found to be associated with asthma prevalence (24). The global prevalence of asthma increased from 11.1% to 11.6% in children aged 6–7 years and from 13.2% to 13.7% in those aged 13–14 years in a 5-10 year period up to 2003 (25). In the USA, asthma prevalence has been steadily increasing from 2001 to

2010 (26), followed by a plateauing and a possible decline since 2013 (27).

Patients' sex influences the epidemiology of asthma and asthma exacerbations. Paediatric studies on asthma show a male predominance, with about 60% of children affected by asthma or asthma exacerbations being male. In adulthood, this sex ratio changes to a dominance of female patients with asthma and increased average symptom scores in females (28). Sex hormones play a role in this difference through both immunological and non-immunological pathways that are not yet completely understood (29).

Social and economic factors influence the prevalence of asthma and the control of asthma and its exacerbations. In the USA, racial disparities persist between African American and Non-Hispanic White children with asthma (27, 30, 31).

IV. Outcome Definitions in Asthma Research

Lack of uniformity in outcome definitions and in the assessment of exacerbation outcomes and asthma control in general, have made prior comparisons and meta-analysis of data from asthma trials difficult. Defining a single primary outcome in pediatric asthma studies is complex. In 2009, a group of experts form the American Thoracic Society/European Respiratory Society defined a list of core-health outcomes that had to be present in asthma research (15). Similarly, a wide consortium of governmental and nongovernmental organizations held an Asthma Outcomes workshop in 2010 (32). In a 2012 National Institutes of Health institutes and other federal agencies workgroup paper, a proposal was made on how an asthma exacerbation should be assessed to use as a standardized outcome in asthma research (17). The presence of an exacerbation defines insufficient and inadequate asthma control and the goal of asthma treatment is to prevent exacerbations. Once the exacerbation has started, the severity may be assessed by

the need or not to present to the emergency department, receive rescue oral steroids and be hospitalised. Hospitalization is a clinically important outcome, the main outcome of interest for patients and one of the core outcomes in clinical asthma research (33) and mainly represents those patients with ED treatment failure. Defining ED treatment failure also requires taking into account patients that will stay for prolonged duration in the ED, due to health care organization and utilization restrictions, including the often occuring unavailability of hospital beds. Early return to the ED after initial discharge is also a measure of ED treatment failure. All previously mentioned elements should preferably be captured in a composite measure of ED treatment failure. Asthma morbidity and costs are mainly due to acute health care utilization, in particular hospital admissions related to asthma exacerbations. Lung function tests and the use of validated composite scores, diary measures using validated diary questions and quality of life scores are alternatives or can be used in parallel, including more patient oriented outcomes.

Asthma mortality in children is low, estimated to be 0.0-0.7/100 000 (34) and is used in prevalence studies, but is very rare or absent in most clinical trials.

V. The role of respiratory pathogens in asthma

Respiratory pathogens – bacterial and viral – are associated with asthma, in various ways. First, they probably play a role in the etiology and disease onset of asthma and, furthermore, respiratory infections are also known to trigger asthma exacerbations.

The complete picture of the contributing factors leading to asthma inception remains unknown. The current hypothesis is that interaction between environmental and pathogen exposure, immune components, and genetic susceptibility, may lead to the inception of asthma in a patient. Studies have shown that exposure to specific respiratory pathogens plays a role in the inception

of asthma. Respiratory syncytial virus (RSV) and rhinovirus (HRV) infection in infants and young children were both associated with an increased risk of developing asthma at age 6 years (35, 36). More recent research also found that asthma is associated with changes in the airway microbiome (37). High microbial exposures to certain bacteria have on the other hand been related to a decreased risk of asthma, through shaping of the innate immune response, as shown both in humans and mice, using models of differential microbial exposures on farms (3).

Both viral and bacterial respiratory pathogens are known triggers of asthma exacerbations in patients with pre-existing asthma (35, 38, 39). In children, the majority of exacerbations are triggered by viral pathogens and respiratory infection. The exacerbation is a reaction to the microbial stimuli, arising from a mediated innate and acquired immune response to pathogen exposure in the asthmatic host (40). Respiratory pathogens have a local effect on respiratory epithelial cells, induce and increase the release of inflammatory mediators and cause changes in the neural control of the airways (41) leading to increased airway inflammation. The mechanisms and pathogenesis are thus related to epithelial cell destruction, induced pro-inflammatory cytokine production and T-helper type 2 cell induction. These mechanisms of airway inflammation and obstruction may be pathogen-specific (42). With the high prevalence of HRV infections in children with asthma exacerbations, in vitro studies have mainly investigated the pathogen-specific effect of HRV on airway epithelial cells and their immunologic response (43).

Moreover, atopy seems to be also an important player in asthma exacerbation initiation and progression, presumably due to interaction with respiratory pathogens. Extensive literature has emerged on the interaction between respiratory viral infection and allergic sensitization. Clinical studies found a positive interaction between atopy and viral pathogens in the onset of asthma exacerbations (44, 45). Whether this interaction leads to differences that are clinically significant between infected and non-infected children once an asthma exacerbation has established, remains

unclear. Murray et al. (46) have shown that atopy and HRV infection is associated with an increased risk of hospital admission, comparing children with an asthma exacerbation to those with stable asthma. Kantor et al. investigated interaction with atopy and found an increased length of active treatment in HRV-infected children with interactions between rhinovirus infection and allergen-specific IgE levels that help to explain additional inter-individual variation in disease manifestations (47). Although there is existing literature on the prevalence of respiratory pathogens in asthma exacerbations, summarized data on the association between respiratory pathogens and clinical important outcomes in children with an asthma exacerbation are lacking.

VI. Burden of respiratory pathogens in children with asthma exacerbations

1. Prevalence of respiratory pathogens in children with asthma exacerbations

It is said that 60-80% of asthma exacerbations (35, 39), or up to 80-85% in the landmark study by Johnston et al. (38), are triggered by respiratory pathogens. Their prevalence depends on the patient's age, season of presentation and the type of patients included in the various studies (community, emergency department or hospitalized).

HRV has been identified as the most prevalent pathogen in children presenting with an asthma exacerbation. Up to 80% of children with a positive laboratory diagnosis for a respiratory pathogen, are positive for HRV (48). Individuals with asthma are not more frequently infected in their upper respiratory tract with HRV, but have more frequent infections leading to lower respiratory tract involvement (49). HRV is a member of the *Picornaviridae* and more than 101 serotypes have been identified. HRV is classified in species A and B and the more recently discovered species, HRV-C (50, 51).

Papadopoulos et al. (52) summarized the current knowledge on respiratory pathogens prevalence in children with an asthma exacerbation from studies over a 10-year period until 2010. In that study, RSV was found as the second most common pathogen, with a prevalence from 2%-68% (median 19%) in infants and pre-schoolaged children with an exacerbation, while parainfluenza virus (PIV) was found in 4%-12% of exacerbations in the youngest age group. In a more recent review on PIV (53), it was described as the third most common pathogen in viral-induced exacerbations. Enterovirus was found in 12%-25% of young children. Human enterovirus 68 (EV-D68) has been described since 1967, but received only limited attention until 2014. Diagnosis of EV-D68 is not included in regular respiratory diagnostic panels and it was only found to be associated with small outbreaks (54). A 2014 outbreak, as reported by Midgley et al. (55) renewed the interest in the virus given the severe respiratory symptoms in infected asthmatic children. Influenza virus was found with a prevalence of 1 to 20% in children with acute asthma exacerbation. Bocavirus, Coronavirus and Adenovirus ranged from 7.5%-19%, 0%-5% and 1.5%-8% respectively.

Mycoplasma is the number one cause of atypical pneumoniae with high prevalence in older children. Atypical bacteria, *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* have been found in 4.5% to 12.4% and 1.6% to 5% (42, 56-58) of children presenting with an asthma exacerbation, respectively.

2. Diagnosis of respiratory pathogens

Diagnosis of specific respiratory pathogens, like influenza or RSV, based solely on clinical symptoms is difficult because their manifestations are variable and non-specific (59). Results of diagnostic tests are required to confirm the presence of a specific pathogen, to guide clinical

management or to retrieve epidemiological indicators (60). For decades, diagnostic tests for respiratory viruses were based on the identification of the virus in a patient's respiratory secretions by virus isolation in culture. This technique has been the gold-standard, but is labor and time consuming, with turn-around times of two to 10-days, reducing its utility for patient management. More importantly, viral culture has limited sensitivity leaving some respiratory infections undiagnosed. Molecular techniques, such as reverse-transcription polymerase chain reaction (RT-PCR), have now replaced viral culture as the reference standard due to their very high analytical and clinical sensitivity and specificity (61, 62). Multiplex testing makes joint diagnosis of a large set of potential pathogens possible. This led to a larger interest in syndrome-based microbiological diagnosis with the simultaneous diagnostics of pathogens of viral, bacterial and sometimes parasitic origin known to cause similar clinical pictures (63). Multiple respiratory panels have become commercially available and RT-PCR is now widely available in clinical practice. The use of RT-PCR facilitates detection of outbreaks (seasonal and sporadic) and rapid diagnosis has the potential to allow for prompt hospital infection control measures (64), to reduce ancillary diagnostic studies (65-69) and reduce the unnecessary administration of antibiotics (65, 66, 68-70).

In clinical practice, RT-PCR's turn-around time for results are often longer than the ~2 hours of analytical time required to run the assay. Specimens need to be sent to specialized laboratories where testing is typically performed in batches. Pathogen identification is thus often not available for the first line management of the potentially infected patient, unless laboratory diagnosis is re-organized. An alternative are rapid diagnostic tests, with possible use at the point-of-care (POC), which results are often provided in less than 30 minutes and have the potential to directly influence patient management. Rapid tests of the older generation were shown to have decreased sensitivity that limited their use(71). Newer generation tests with automated readers added to the

viral antigen detection immunoassays or POC tests based on rapid nucleic acid amplification (NAATs), have improved sensitivity while maintaining specificity and are now increasingly used as an alternative or in combination with RT-PCR in certain clinical settings. Rapid tests are however limited to the simultaneous detection of only 1 or 2 specific respiratory pathogens.

For research purposes, sequencing has led to the identification of specific species and subtypes of respiratory pathogens. These technological advances have improved our knowledge on species-specific incidence and make strain-specific investigation possible.

Chapter 2: Rationale and thesis objectives

Rationale

Asthma is defined as a chronic inflammatory disorder of the airways and is the single most common chronic disease in childhood (1), both in developing and developed countries. Exacerbations contribute to the largest burden of disease. A significant number of patients respond suboptimaly to the standardized treatment of corticosteroids and severity-specific bronchodilators. Respiratory pathogens and infections are the main trigger of asthma exacerbations in children, with 60-80% of these related to a respiratory pathogen. In vitro studies have investigated pathogen-specific effects on the airway inflammation and activated immune cascades. Still, the actual burden of various respiratory pathogens and their individual impact on the clinical evolution of the exacerbation and response to treatment as administered in the emergency department (ED), remain unknown. Understanding the role of a particular respiratory pathogen in children with an exacerbation would help to evaluate the utility of infection prevention, timely pathogen identification at presentation using molecular diagnostics or rapid diagnostic tests, pathogen-adjusted treatment, and follow-up regimens.

Thesis Objectives

This manuscript-based thesis contains two manuscripts and has the following objectives.

1. To describe the peer-reviewed evidence of the association between the presence of specific respiratory pathogens and clinical relevant outcomes for asthma exacerbation in the pediatric population, by performing a systematic review of the published literature,

2. To describe the prevalence of Adenovirus (B, C and D), Coronavirus (229E-, HKU1, NL63, OC43), Enterovirus (A, B, C, D, including EVD68), Influenza virus (A,B), Parainfluenza virus

((1,2, 3 and 4), human metapneumovirus (A and B), respiratory syncytial virus (A and B), rhinoviruses (A, B and C), *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* in children with an acute asthma exacerbation presenting to emergency departments across Canada, using the data from a Canadian prospective cohort (DOORWAY) study of almost 1000 children with a moderate or severe asthma exacerbation,

3. To analyse the respiratory pathogen-specific risk of (i) severe versus moderate exacerbation on presentation and of (ii) emergency department management failure (failure of *per protocol* treatment with corticosteroid and severity-specific bronchodilators leading to hospitalization, prolonged stay at the emergency department or admission or prolonged stay after re-consultation within 48 hours of initial ED discharge) in the DOORWAY cohort.

Chapter 3: Systematic Review of the literature: Association between respiratory pathogens and clinical outcomes: severity, health care utilization, treatment response and morbidity and mortality, in children with an asthma exacerbation

In this first manuscript, a systematic review, we aimed to summarize and critically analyze the available evidence from studies reporting on the association between laboratory-diagnosed respiratory pathogens in children presenting with an asthma exacerbation, and one or more clinical relevant outcomes namely, the severity of asthma exacerbation, health care utilization, treatment response, morbidity, and mortality. Our objective was to compare short-term clinical outcomes of asthma exacerbations in children (i) with and without an associated respiratory pathogen in general and (ii) with and without specific pathogens and co-infection. Our thorough literature search led to the inclusion of 28 articles. Data were extracted for all the respiratory pathogen exposures and the *a priori* formulated clinically important and informative outcomes, resulting in reporting on a total of 112 pathogen exposure-outcome associations. A validated quality assessment tool was used to investigate the risk of bias per exposure-outcome association and found high quality evidence lacking for most of the associations. All data are presented per outcome domain in table format. The written results section and discussion is limited to associations assessed as a mild to moderate risk of bias, the only findings contributing to the strength of evidence. The manuscript is presented as submitted to *The Pediatric Infectious Disease Journal.*

Systematic Review

Respiratory Pathogens and Exacerbation Outcomes in Asthmatic Children: A Systematic Review

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Abbreviated Title: Respiratory Pathogens and Asthma Exacerbation Outcomes

Running Head Title: Pathogens and Asthma Exacerbation

Abstract

Background: In asthmatic children, respiratory pathogens are identified in 60-80% of asthma exacerbations, contributing to a significant burden of illness. The role of pathogens in the clinical evolution of exacerbations is unknown.

Objective: Systematically review the association between the presence of pathogens and clinical outcomes in children with an asthma exacerbation.

Methods: PubMed, EMBASE, BIOSIS and the Cochrane Central Register of Controlled Trials were searched up to October 2016. Studies reporting on respiratory pathogen exposure and a clinical outcome: exacerbation severity, healthcare (HC) utilization, treatment response or morbidity, were eligible and screened in duplicate. The Risk of Bias In Non-Randomized Studies of Interventions tool was used for quality assessment.

Results: Twenty-eight observational studies (4224 children) reported on 112 different associations between exposure to any pathogen (n=45), rhinovirus (HRV; n=34), atypical bacteria (n=21), specific virus (n=11) or bacteria (n=1) and outcomes of exacerbation severity

(n=26), HC utilization (n=38), treatment response (n=19) and morbidity (n=29). Restricting only on comparisons with moderate risk of bias, we observed an association between HRV and higher exacerbation severity on presentation (regression p-value 0.016) and between the presence of any pathogen and emergency department treatment failure (OR 1.57, 95% CI 1.04-2.37). High quality evidence on effect on morbidity or HC utilization is lacking.

Limitations: The heterogeneity of published data precluded data aggregation. The lack of good quality data limited the strength of evidence.

Conclusions: Further research on the role of pathogen-treatment interaction and outcomes is required to inform the need for point-of-care, real-time testing for pathogens.

Abbreviations:

CI – confidence interval

ED - emergency department

ENV-D68 - enterovirus-D68

GINA - Global Initiative for Asthma

HC – healthcare

HRV – humane rhinovirus

INF – influenza

NIH - National Institutes of Health

OR - odds ratio

RCT - randomized controlled trials

RSV - respiratory syncytial virus

1 Introduction:

Asthma is a chronic inflammatory disorder of the airways and is the single most common chronic disease of childhood (1, 2). Asthma morbidity and costs (3) are mainly due to acute exacerbations and associated hospital admissions. Respiratory pathogens along with host-pathogen interactions play a role in the inception of asthma (4). Respiratory pathogens, both viral and bacterial, are also known triggers of asthma exacerbations (5, 6). Other endogenous and exogenous stimuli (7) that may trigger or worsen asthma symptoms, include allergens, tobacco smoke, exercise and stress (8). In children, 60-80% of exacerbations are associated with viral pathogens and respiratory infections (4, 5). Moreover, multiplex real-time polymerase chain reaction (RT-PCR) has enhanced the detection of a larger number of different pathogens, given its improved sensitivity, while sequencing has allowed for the identification of specific subtypes (9), resulting in a renewed interest for studying specific pathogens and their role in asthma exacerbations.
Human rhinovirus (HRV) has been identified as a prevalent pathogen in children with asthma exacerbations that follows seasonal peaks (10). To understand this phenomenon further, studies have investigated the pathogenicity and interaction of HRV with the epithelial cells of the respiratory tract and with immunological pathways (11-13). The role, pathogenicity and impact on clinical severity of individual HRV subtypes, including the recently discovered HRV-C, in children with and without underlying asthma, are still debated (14-16). Moreover, recent outbreaks of specific pathogens, such as enterovirus-D68 (ENV-D68) have been linked with severe respiratory complications in children with asthma (17-19). The role of influenza (INF) and its association with asthma exacerbation severity and health care utilization remains controversial (6, 20). The atypical bacteria, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have been associated with exacerbations; however, their impact on clinical evolution is not clear (21).

There has not been any systematic evaluation of the evidence related to the strength of association, if any, between the presence of a respiratory pathogen and the severity of asthma exacerbation as well as its clinical evolution. In addition, the actual importance of specific respiratory pathogens and their impact on the clinical picture, exacerbation evolution and response to treatment (steroids and bronchodilators) remains unclear. Understanding the role of a particular respiratory pathogen in children with an exacerbation would help to evaluate the utility of infection prevention, pathogen identification at presentation, pathogen-adjusted treatment, and follow-up regimens.

In this systematic review, we aimed to summarize and critically analyze the available evidence from studies reporting on the association between respiratory pathogens with one or more clinical relevant outcomes namely, the severity of asthma exacerbation, health care utilization, treatment response, morbidity, and mortality. Our objective was to compare short-term clinical outcomes of asthma exacerbations in children with and without an associated respiratory pathogen.

2 Methods:

We used the PRISMA guidelines (22) for the design, performance and reporting of this review. A research protocol using the PRISMA-P guidelines (23) specifying our methods was developed in advance.

1. Eligibility Criteria: Types of studies, participants, exposures and outcomes :

Studies providing original data examining the association between the presence of respiratory pathogens and asthma exacerbation, in children less than 18 years of age, were included. Quantitative study designs including all types of observational studies (cohort, case-control, cross-sectional and longitudinal) and randomized controlled trials (RCT) were considered. Case

reports, case series, ecological studies and studies with only an abstract available were excluded. Studies investigating the association of respiratory pathogens and the inception of asthma and studies only aiming to determine infection/viral incidence in asthmatics were also excluded.

Participants were restricted to children with pre-existing and diagnosed asthma. Studies of children with 2 or more previous wheezing episodes were eligible. This was based on the criterion validity in predicting the presence of asthma (24), which has been proposed by the Canadian Thoracic and Paediatric Society(25), and used in previous published peer-reviewed literature(26, 27).

The presence of respiratory pathogens - bacterial and/or viral - or one specific respiratory pathogen, had to be investigated systematically in the study population. Studies looking at sensitization to fungal elements were not within the scope of this review. PCR is the current gold standard in the diagnosis of respiratory pathogens from the upper respiratory tract, replacing viral and bacterial cultures. The use of PCR has led to wider diagnosis of the presence of (multiple) respiratory pathogens (9, 28). In practice, less sensitive ELISA tests and rapid tests have also been widely used to diagnose respiratory pathogens. Yet, for our systematic review, all diagnostic methods, including serology testing, were accepted.

Defining a single primary outcome in pediatric asthma studies is complex. In 2009, a group of experts form the American Thoracic Society/European Respiratory Society defined a list of core-health outcomes that had to be present in asthma research (29). Similarly, a wide consortium of governmental and nongovernmental organizations held an Asthma Outcomes workshop in 2010 (30). We used the outcomes agreed upon in both documents as guidance for the evaluation of asthma outcomes in the studies included in our systematic review. The outcomes were grouped under: 1) Severity of the asthma exacerbation, 2) Healthcare utilization, 3) Response to treatment, and 4) Other indicators of morbidity and mortality (Table 1). Studies were included if they

reported absolute numbers (means, proportions), inference testing with absolute numbers or effect measures (odds ratio (OR), relative risk, or model coefficients) with confidence intervals. Each study could provide more than one exposure-outcome comparison for assessment.

2. Search Strategy and Study Selection:

An electronic search strategy was developed in collaboration with a librarian (GG) with expertise in knowledge synthesis. PubMed, EMBASE, BIOSIS and the Cochrane Central Register of Controlled Trials (CENTRAL) were systematically searched without date or language restrictions. A primary search was done on December 11th 2015 with an update on October 19, 2016. Our search strategies are available in Appendix 1. Four domains were searched: asthma, child, respiratory pathogens, and outcomes. Citations of included articles were hand searched. EndNote (Version X 7.5, Clarivate Analytics, Philadelphia, PA) was used for the merging and de-duplicating of the articles. Distiller (Distiller SR v2 - Evidence Partners, Ottawa, Ontario, Canada) was used for title and abstract screen, full text screen and data extraction. Articles were restricted to English and French. Two reviewers (HK and JM) independently screened citations (titles and abstracts) identified by the search strategy. Potentially relevant articles were retrieved in full and assessed for eligibility for inclusion by the 2 independent reviewers. Disagreement was resolved by consensus.

3. Risk of Bias Assessment and Data Collection:

We used the updated and validated Cochrane Collaboration's "Risk of Bias In Non-Randomized Studies – of Interventions" tool (ROBINS-I) (31) to assess the risk of bias for each outcome within the studies. The various bias domains: bias due to confounding, patient selection, exposure classification, missing data, outcome measurement, and selective reporting of results were

explored per outcome within each study. We considered age as a critical confounder for which adjustment was required. Each study was judged to be at low, moderate, serious or critical risk of bias for each domain and overall, for each specific outcome. Quality assessment was not a judgement of the overall methodological quality of the study, as our objectives differed from the initial study in many cases, but rather of how well the measurement of each exposure-outcome association reflected the association between the investigated respiratory pathogens and the chosen outcome. Data and quality assessments were extracted using a pre-piloted data extraction form by a single reviewer (JM), with quality control performed by a second reviewer (HK). Study authors were contacted by email when needed to address issues of insufficient clarity or missing information.

4. Data Analysis:

Descriptive statistics were used for analysis. Summary tables present the characteristics for each study and outcomes for each exposure-outcome comparison, with more than one comparison per study. Outcomes are presented as means and proportions in respiratory pathogen-exposed and unexposed groups. P-values are reported when no other effect measures and/or confidence interval was reported, acknowledging its limited value in assessing the clinical significance of the findings, the association strength, or the presence of a causal effect (32). Conclusions were based only on associations of interest assessed at low or moderate risk of bias. Only outcomes with non-critical overall risk of bias are included in the detailed description of the outcome domains. Given the heterogeneity of outcome domains and measures assessed, a meta-analysis could not be carried out. Data were analyzed using R version 3.2.1 (www.r-project.org) and STATA 13 (StataCorp. College Station, TX).

3 Results:

1. Search Results:

A total of 14,424 records were identified in December 11th, 2015 with an updated search (October 19th, 2016) yielding an additional 906 records. A total of 10,329 unique records were screened for eligibility by title and abstract with 232 assessed as full texts. Finally, 28 studies were retained for inclusion(26, 33-59). The flow diagram (Figure 1) details reasons for exclusion. Reference search of included studies did not identify any additional records.

2. Characteristics of the included studies:

Of the 28 included studies, there were 20 cohort, 3 cross-sectional, 4 case-control studies and 1 RCT. All studies were published in English; they were conducted in Australia (n=4), China (Hong Kong) (n=2), France (n=3), Japan (n=3), Turkey (n=2), USA (n=6) and (n=1) in the countries Argentina, Canada, Colombia, Egypt, Finland, India, Portugal and Taiwan. All studies, but one published in 1970, (39) were performed between 1999 and 2016. Study characteristics are described in Table 2.

In 15 of 28 included studies, we only included a portion of the original study population to focus on children with acute asthma and when indicated, we excluded asthmatic patients without an acute exacerbation, non-asthmatic control groups, and adults. The total number of included participants was 4,224, with almost all studies (24/28) including, but not limited to, patients under the age of 5 years. The average proportion of males was 60.6% in studies reporting participants' sex. Asthma diagnosis leading to participant inclusion was primarily (32%; 9/28) defined using guidelines-defined criteria (Global Initiative for Asthma (GINA), National Institutes of Health (NIH), British Thoracic Society and American Thoracic Society). The main

criteria used to diagnose an asthma exacerbation were clinical symptoms (9/28) and the GINA guidelines (8/28).

Twenty-one of 28 (75%) studies used PCR to detect the presence of a respiratory pathogen in the upper respiratory tract; 24 investigated viral pathogens, 11, atypical bacteria and 2, bacteria. Among viral pathogens, the most frequently investigated was respiratory syncytial virus (RSV) (23/28 studies). Fifteen studies investigated the presence of HRV, with subtyping being completed in 4 studies. Detailed characteristics for each study are presented in Table 3. Nineteen studies investigated the severity of asthma exacerbation, 19, the use of the healthcare system, 8, the response to treatment and 16, the morbidity and mortality of children with and without any respiratory or a specific pathogen. In total, 112 exposure-outcome associations were investigated.

3. Quality and risk of bias assessment of included studies:

Figure 2 summarizes the risk of judgement bias per outcome domain as extracted from 112 comparisons. The quality assessment is presented per outcome domain and per bias domain, with most single studies contributing to more than one outcome within and between outcome domains. In Appendix 2, the detailed risk of bias assessment is presented for each exposure-outcome assessed. None of the outcome assessments had a low overall risk of bias, 7 had a moderate, 85 a serious, and 20 had a critical risk of bias. Lack of investigation of confounding, selection bias, and multiple testing with reporting bias were the major problems with the quality of the investigated exposure-outcome associations.

4. Exposure-Outcome Results:

Of the 112 exposure-outcome comparisons, the primary exposure was the presence of any

pathogen in 45 (40%) comparisons, any type of HRV in 34 (30%), atypical bacteria in 21 (19%), specific viruses in 11 (10%) and only one looked at the presence of any bacteria. Detailed results are organized per outcome domain in Appendix 3.

In 34% (38/112) of associations, the relationship between exposure and health care utilization was investigated. In 26 % (29/112) of associations, the clinically relevant outcome was morbidity or mortality, in 23% (26/112), the outcome was exacerbation severity, and 17% (19/112) had response to treatment as the investigated outcome. The variety in exposures and outcomes precluded data aggregations. Very few outcomes were assessed using methods others than inference testing, and the presence of an association was often only suggested by the presence of a so-called statistical significant p-value (p-value <0.05). In the following section, we described the data derived from the 7 associations considered at moderate risk of bias and pertaining to 112 comparisons, with added evidence from serious non-critical risk of bias where appropriate.

3.4.1 Association between presence of respiratory pathogens and exacerbation severity

When stratified by exposure, 5 comparisons were in favour of an association between HRV and increased severity of the exacerbation (35, 40, 46) and 2 were not (45, 47), based on statistical significant differences (including p-value <0.05). In the only published article at moderate risk of bias for this outcome (40), HRV-C was associated with a higher severity score on presentation compared to HRV-C negative patients presenting to the emergency department (ED): the mean score in patients exposed was 10.9 (95%CI 10.0-10.9) compared to 9.4 (95%CI 8.7-10.2) in non-exposed (linear regression coefficient adjusted for age and sex, p-value=0.016). In this study, a modified NIH severity score (60) was used (range: 0 to 15), with a score between 8 and 11 being defined as a moderate exacerbation and between 12 and 15, indicative of a severe exacerbation. This positive association also remained when HRV-C positive participants were compared to those infected with other viral pathogens (score 10.4; 95%CI 8.7-10.1) and to HRV-A and B

subtypes (score 9.4; 95%CI 8.7-10.2) (40).

When comparing asthma exacerbation severity at presentation in patients harbouring any pathogen vs. pathogen-negative ones, only one (33) of five comparisons (41, 47, 54, 58) favoured a positive association . The presence of atypical bacteria (36, 42) was not associated with higher severity scores. There is insufficient or only low quality evidence for an association between any pathogen and hypoxia (48) or the need for, (46) or duration of, oxygen therapy (37, 43, 58).

3.4.2 Association between the presence of respiratory pathogens and healthcare utilization

No comparison with a low or moderate risk of bias was available for healthcare utilization, such that we cannot make any sound conclusion on the association between pathogens and healthcare use. Six of 30 comparisons at serious risk of bias investigated the association of respiratory pathogens and the need for hospital admission (26, 33, 42, 46, 54); another six comparisons investigated the presence of HRV or of any pathogen and its association with length of hospitalization (44, 46-48, 58) and four studies explored the effect of atypical bacteria (36, 37, 42, 43) on the length of hospitalization. There is only low quality evidence for the association of intensive care admission, length of stay, or mechanical ventilation with RSV or HRV(46, 57), but not with pathogen exposure in general (33) or with atypical bacterial infection (43, 61). Clinical deterioration leading to a return visit or readmission was investigated in 4 articles (26, 44, 55, 61).

3.4.3 Association between the presence of respiratory pathogens and treatment effect

Only one study with a moderate risk of bias was identified with regards to treatment response. This study was conducted in children with a moderate or severe asthma exacerbation who were treated according to evidence-based guidelines in the ED (26). The authors observed, as their primary outcome, an association between the presence of any respiratory pathogen and an increased risk of ED treatment failure (OR 1.57; 95%CI 1.04-2.37), after adjusting for fever,

baseline PRAM clinical score (62), oxygen saturation, study site and symptoms between exacerbations. No increased odds of treatment failure was observed when comparing HRV-positive to HRV-negative patients in this study (OR 0.96; 95%CI 0.67-1.39). The latter comparison was however assessed as having a serious risk of bias investigation. As secondary outcomes, the authors reported an association between the presence of any pathogen and prolonged duration of symptoms following discharge from the ED/hospital despite oral corticosteroid treatment. Pathogen presence was also associated with increased length of active treatment in the ED and length of rescue ß2-agonist use over the next 10 days. Other evidence investigating an association between the presence of a pathogen and treatment response to bronchodilators (46), bronchodilator use (43, 56), need of steroid treatment (54, 61), and duration of steroid treatment (47) was assessed at high risk of bias.

3.4.4 Association between the presence of respiratory pathogens and morbidity/mortality

High quality data are lacking on the association between pathogens and other indicators of morbidity. This includes the absence of high quality evidence for an association between lung consolidation or infiltrate and the presence of any pathogen (33, 58, 61), the presence of atypical bacteria (36, 37, 43, 55) or bacteria (53) at time of exacerbation. Duration of symptoms (33, 44, 48, 54, 58, 63), symptom scores (35), respiratory function evaluation (46, 48) and quality of life (26, 35) were investigated but comparisons were at a serious risk of bias leading to inconclusive results.

4 Discussion:

The large quantity of different exposures and comparisons, the heterogeneity of outcomes, and the risk of bias assessed as high or critical in most exposure-outcome comparisons in the 28

identified studies, prevent firm conclusions on the potential impact and magnitude of effect of respiratory pathogens on asthma exacerbation outcomes. Our systematic review concludes to a possible association of HRV and, more specifically of subtype C, with more severe asthma exacerbation at ED presentation. It also established a poorer treatment response to maximised bronchodilators and systemic corticosteroid in the ED in presence of any respiratory pathogen but not specifically HRV, in children with a moderate to severe exacerbation. However, the impact of specific respiratory pathogens or pathogens in general on health care utilization and other indicators of morbidity is not clear. Our conclusions are based on only two studies providing an outcome assessment with a moderate overall risk of bias (Bizzintino et al. (40) and Ducharme et al. (26)).

Specific respiratory pathogens are suspected to influence clinical presentation of acute asthma exacerbations (5, 64). There was however an insufficient number of adequately powered high quality studies in our review to provide conclusive evidence on pathogen-specific effects, with the exception of a possible effect of HRV on exacerbation severity. HRV and its subtypes are highly prevalent in pediatric asthma exacerbations (65), with a higher prevalence in asthmatic children presenting with an exacerbation than those without exacerbation (66). Lower respiratory tract symptoms persist for longer duration and are more severe in asthmatic than in non-asthmatic children (10, 67). Bizzintino et al. (40) described that children infected with HRV and particularly HRV-C had a higher asthma severity (by one-point on the average clinical severity score) at presentation. Perhaps, a defect in the HRV-induced innate immune response may be of importance leading to more severe infection, particularly with HRV-C subtype. Indeed, interferon type I and III response are modified (68, 69) in asthmatic children infected with HRV. In addition, there is evidence that HRV leads to an aberrant adaptive immune response through

rapid recruitment of circulating CD4 and CD8 (70). In asthmatics, a more pronounced expression of cytokines associated with Th2-lymphocytes is postulated. Accordingly, the observed positive association between HRV and asthma severity might be explained by the impaired immune response to respiratory pathogens, and more specifically to HRV, which drives exacerbation development and presentation (71).

Extensive literature has emerged investigating the interaction between respiratory viral infection and allergic sensitization. Clinical studies reported a positive interaction between atopy and viral pathogens in the onset of asthma exacerbations (72, 73). Whether this interaction leads to clinically important differences between infected and non-infected children once an asthma exacerbation has established, remains unclear. The only study investigating the interaction between atopy and viral pathogen that was included in our review reported an increased length of active treatment in HRV-infected children (46). The risk of bias in this study was however assessed as serious.

Although several reports have suggested a role of HRV co-infections and infection with multiple pathogens on the severity of exacerbation (74, 75), studies included in our review did not provide sufficient data to conclude on the impact of co-infections on clinical outcomes. RSV was the most sought exposure and the second most frequent pathogen after HRV, however its specific role with regards to exacerbation severity and clinical exacerbation evolution has not been investigated extensively.

In their large prospective cohort, Ducharme et al (26) showed that the presence of a respiratory pathogen was associated with increased ED treatment failure (prolonged ED stay, hospitalization

and relapse). This suggests an infection-mediated altered response to maximized bronchodilator and oral corticosteroid therapy, which are the cornerstone of ED treatment of moderate to severe exacerbations in children (8, 76). The decreased response was not attributable to HRV. However, with only one published study, there is a need for more data to further explore the clinical importance of specific respiratory pathogens associated with exacerbations and their treatment response.

This review has several limitations. First, we could only comment on associations and not on any causal role of respiratory pathogens, on clinical outcomes of interest. The presence of a pathogen, when identified by PCR, cannot differentiate between dead pathogens and actively replicating infectious pathogens nor determine whether the exacerbation was indeed triggered by the pathogen. Kling et al. (77) reported that over 40% children with a PCR- positive specimen for HRV during an asthma exacerbation still had detectable HRV by PCR 6 weeks after the exacerbation, suggesting the possibility of prolonged shedding. Alternatively, Engelmann et al (78) proposed that re-infection, rather than pathogen persistence, may better explain this finding. In clinical practice, there is no way to differentiate colonization from infection. In all included studies, misclassification of some patients colonized with a respiratory pathogen as patients with an exacerbation triggered by the identified pathogen, is likely. Second, most studies were too small and lacked sufficient power to firmly conclude on the potential effect of less prevalent pathogens on various exacerbation outcomes. Third, given the differences in outcomes and effect measures reported in the different studies, it was not possible to investigate a potential publication bias in any formal way. Finally, the poor methodological quality of most included studies (85/112 and 20/112 comparisons at serious and critical risk of bias) prohibits firm conclusions. The lack of formal statistical investigation and adjustment for confounding variables

were the main methodological issues. The risk of selection bias is important when included children where all hospitalized or presenting to the ED. Selective reporting of the numerous possible exposure-comparators and outcome associations was also identified as an important source of bias. Non-significant results were often either not reported or reported but without their estimates of effect.

There is a need for good quality studies that report and adjust for known confounders, with rigorous reporting of all outcomes. The investigation of the interaction between pathogens and between pathogens and atopy, and further investigation of possible differential treatment responses would improve the strength of the evidence. Importantly, it would lead to firmer conclusions regarding the impact of specific pathogens and co-infection on outcomes of interest.

5 Conclusions:

In summary, the evidence suggest a potential association between HRV and higher severity of asthma exacerbations in children presenting to the ED. In children with a moderate or severe asthma exacerbation, the presence of one or more respiratory pathogens is associated with a lower treatment response. While these findings deserve replication, further investigation of the effect of specific pathogens seems appropriate, mainly in light of growing evidence of the importance of specific pathogens such as HRV and HRV-subtype infections. Increased knowledge on host-pathogen and pathogen-pathogen interactions and their role during an asthma exacerbation may enable us to better identify and perhaps manage children with acute asthma at

higher risk of treatment failure. Identified key pathogens could thus be targeted by specific preventive approaches and rapidly identified with point-or-care diagnosis.

6 Acknowledgements

The research project was funded through internal fund

Table 1. Description of Outcome Domains:

1. Severity of asthma exacerbation
• Severity of asthma exacerbation often expressed on severity scale (mild, moderate or severe)
• Hypoxia and/or oxygen therapy need
• Duration of oxygen treatment
2. Health care utilization:
• Need for systemic treatment
• Hospital admission for asthma
• Duration of hospitalization
• ICU admission for asthma
• Duration of ICU admission
• Mechanical ventilation
• Relapse/New consultation
3. Response to treatment
• Length of active treatment
• Duration of symptoms after treatment
• Number of bronchodilator doses needed
• Need for corticosteroids
• Change in lung function following treatment
• Treatment failure
4. Morbidity or mortality
• Death
• Sequelae
• Asthma-related school absenteeism
• Quality of life: CHSA, PAQLQ, pediatric caregiver AQLQ, PedsQL 3.0, Asthma Module PACD (daily diary) (self-perception of health status – symptom free days)
• Duration of symptoms
• Consolidation/pneumonia
• Lung function test

Figure 1. **PRISMA Flow Diagram**

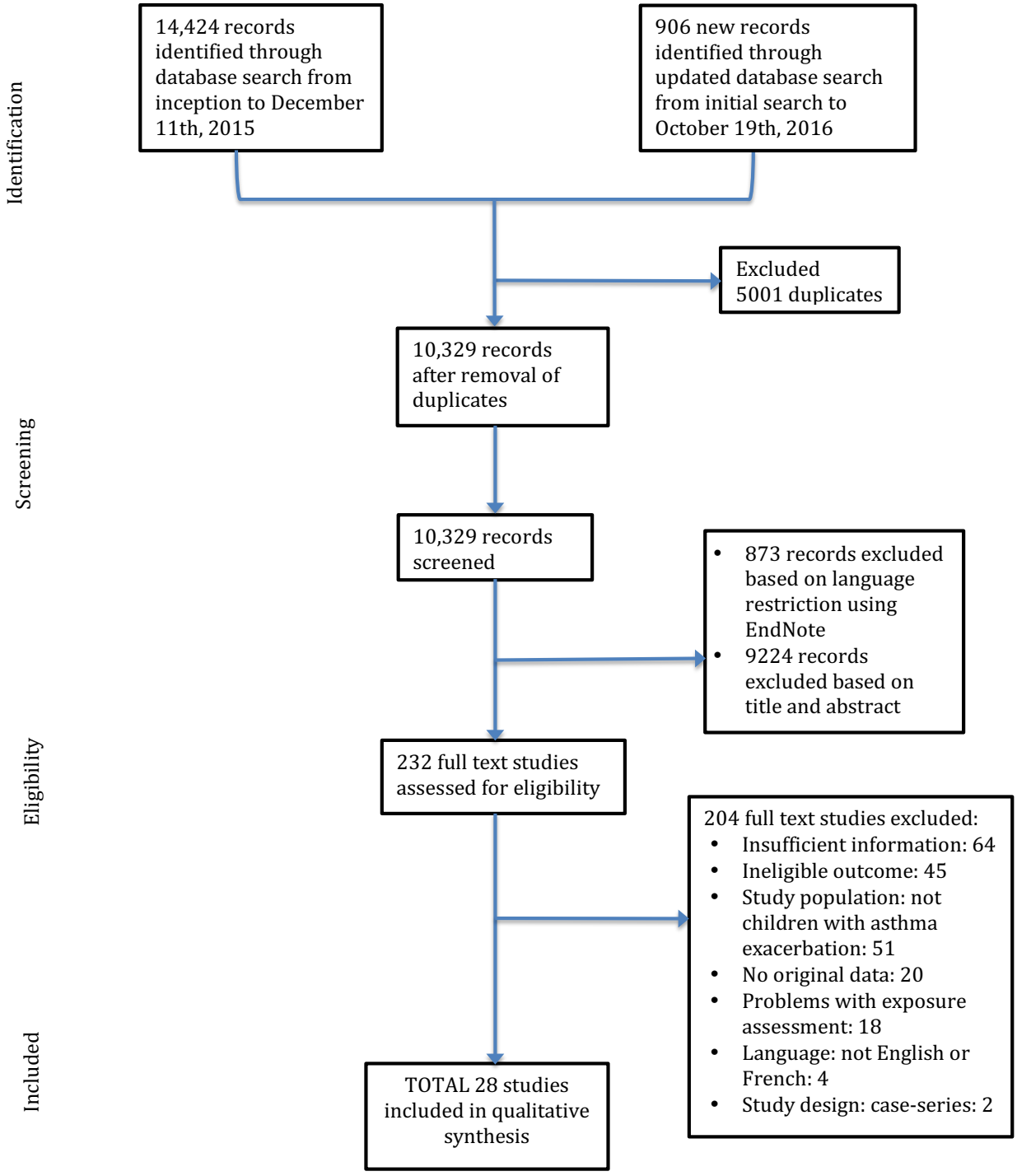

Table 2. Characteristics of included studies (n = 28)

Extracted information		*Number of studies=n (%)*
Study Design	Cohort study	20 (71.4)
	Case-control study	4 (14.3)
	RCT	1 (3.6)
	Cross-sectional study	3 (10.7)
Study season	Year-round	20 (71.4)
	Fall and winter	4 (14.3)
	Fall, winter, spring	1 (3.6)
	Winter, early spring	1 (3.6)
	Not reported	2 (7.1)
Study Setting*	Emergency department	13 (46.4)
	Outpatient clinic	6 (21.4)
	Hospitalized patients	12 (42.9)
	Sub-study of asthma study in community	1 (3.6)
Asthma definition used*	Institutional guidelines#	9 (32.1)
	Physician diagnosis	11 (39.3)
	Hospitalized for asthma	1 (3.6)
	Pulmonary function	1 (3.6)
	Clinical diagnosis	6 (21.4)
	Asthma control medication use	4 (14.3)
	Response to bronchodilator therapy	4 (14.3)
	Recurrent wheezing symptoms (>=3 wheezing episodes including presenting episode)	11 (39.3)
	Patient self-diagnosis of presence of past symptoms – or diagnosis according to parents	1 (3.6)
	Not defined	3 (10.7)
Asthma exacerbation definition*	Clinical symptoms	9 (32.1)
	Institutional guidelines	8 (28.6)
	Physician diagnosis	3 (10.7)
	Asthma medication use	3 (10.7)
	Health service utilization	1 (3.6)
	Not defined	7 (25.0)
Sex participants	% male >50%	21 (75.0)
	%male > 65%	9 (32.1)
	Not reported	4 (14.3)

Exposure measurement*	PCR on respiratory specimen	21 (75.0)
	Point-of-care test on respiratory specimen	2 (7.1)
	Viral culture of respiratory specimen	6 (21.4)
	DFA † on respiratory specimen	5 (17.9)
	IFA on respiratory specimen	5 (17.9)
	Bacterial culture on respiratory specimen	3 (10.7)
	Serology on blood or serum	8 (28.6)
	Molecular typing	2 (7.1)
Industry sponsoring	yes	3 (10.7)
	no	18 (64.3)
	Not reported	7 (25.0)

*not mutually exclusive; #: GINA-guidelines, NIH guidelines, British Thoracic Society guideline, American Thoracic Society; † Direct Fluorescent Antibody

Figure 2. ROBINS-I Risk of bias assessment is presented per outcome domain: severity of exacerbation, health care utilization, response to treatment and morbidity or mortality. The Y-axis shows the proportion of exposure-outcome associations with low, moderate, serious or critical risk of bias within the different risk of bias domains.

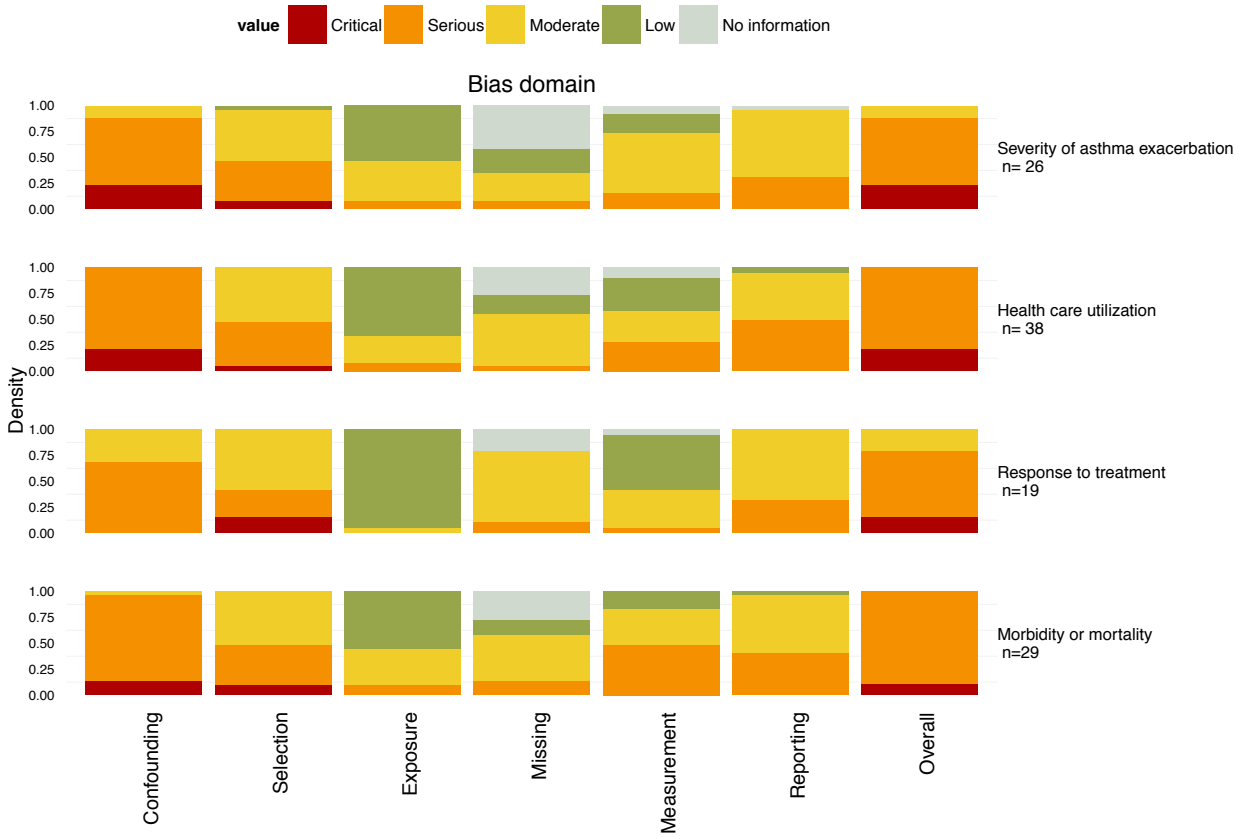

Table 3. Detailed Study Description

Study Primary Author, Year - Country	Study Design	Studysetting‡	Season and Study Duration	Study Definition of Asthma	Study Definition of Exacerbation	Sample Size Included	Age Study Population	Diagnostic Method Pathogen Exposure Diagnosis†	Pathogens Investigated†	Proportion Pathogen Positive n(%)	Most frequent diagnosed pathogen n(%)†	Co-infection n(%)
Akturk, 2016 - Turkey (33)	case-control	ED	winter, early spring; 12/2013 to 04/2014	≥3 episodes of wheeze/dyspnea, clinically improved with beta2-agonist	Clinical symptoms	125	2.5-15y, median 5.5y	RT-PCR	HRV, RSV, hMPV, ADV, HBoV, M pneumoniae, INF-A, INF-B, H1N1, PIV (types 1,2,3 and 4), CoV-NL63, CoV-229R, CoV-OC43, CoV-HKU1, EV, Para-echovirus	106/125 (85%)	HRV 38/125 (30%)	17/125 (14%)
Amin, 2013 - Egypt (34)	cohort	hospitalized patients	year round; 01/2011 to 12/2011	repeated episodes of wheezing, with bronchodilator response and/or on long-term controller therapy	not defined	130	2-12y, mean 2.95y (SD 2.46y)	RT-PCR	RSV, hMPV, ADV, INF-A, PIV	54/130 (42%)	RSV 28/130 (22%)	0/130 (0%)
Arden, 2010 - Australia (35)	cohort	ED	year round; 03/2005-02/2007	>2 episodes of wheeze/dyspnea with doctor diagnosis of clinical response to beta2 agonist	Asthma medication use	51 (total study sample size 78)	median 5.1y (IQR 2.5-7.3y)	RT-PCR with DNA sequencing analysis	HRV-A, HRV-B, HRV-C, RSV, hMPV, ADV, HBoV, INF-A, INF-B, EV, CoV, PIV, Echovirus, Para-echovirus	51/78 (65%)	HRV 41/78 (53%)	20/78 (26%)
Awasthi, 2012 - India (36)	cross-sectional	hospitalized patients	year round; 08/2010 to 08/2011	GINA-guidelines (2009)	GINA guidelines	44	1-12y	serology	C pneumoniae	11/44 (25%)	C pneumoniae 11/44 (25%)	
Bebear, 2014 - France (37)	cohort	ED and hospitalized patients	year round; 03/2007 to 10/2010	GINA-guidelines	GINA guidelines	168	Infected 5.4y (Q1 3.3;Q310.4) vs. non-infected 3.3y (Q1 1.5y; Q3 5.8y)	RT-PCR ; bacterial culture ; serology	HRV, RSV, MPV, ADV, HBoV, INF-A, INF-B, EV, CoV, PIV, M pneumoniae, C pneumoniae	73/168 (44%)	NR	7/168 (4.17%)
Belessis, 2004 - Australia (38)	case-control	ED and hospitalized patients	year round; 02/2000 to 06/2001	diagnostic criteria based on ISAAC questionnaire and National Asthma Council Australia classification	not defined	92	1-16y	RT-PCR ; viral culture ; IFA ; serology	HRV, RSV, hMPV, ADV, M pneumoniae, C pneumoniae, INF-A, INF-B, PIV type 1,2,3	79/92 (86%)	HRV 67/92 (73%)	6/92 (7%)
Berkovich, 1970 - USA (39)	cohort	outpatient clinic	fall, winter; 09/1967 to 02/1968	not defined	not defined	79 (108 ashtma exacerbation episodes)	0.5-16y	viral culture ; bacterial culture ; serology	RSV, ADV, M pneumoniae, INF-A, INF-B, PIV type 1,2,3, Streptococcus pneumoniae, beta-haemolytic streptococci	27/79 patients - 33/108 episodes	INF-A 12/108 episodes (11%)	not clear
Bizzintino, 2011 - Australia (40)	cohort	ED	year round; 04/2003 to 02/2010.	doctor diagnosis of wheezing with increased difficulty of breathing, based on American Thoracic Society definitions, 2009	American Thoracic Society Guidelines	126	2-16 y; mean 6.4y (SD 3.3y)	RT-PCR ; DFA testing on fresh specimen; DNA sequencing analysis	HRV, RSV, hMPV, ADV, HBoV, INF-A, INF-B, PIV (types 1,2,3 and 4), EV, CoV	118/128 (92%)	HRV 112/128 (88%) with HRV-C 76/128 (59 %)	14/128 (11%)
Coleman, 2015 - USA (41)	cohort	not reported	not reported	≥1 of: (1) physician diagnosis of asthma, (2) use of albuterol for coughing or wheezing , (3) use of a daily controller medication, (4) step-up plan including use of albuterol or short- term use of inhaled corticosteroids during illness, (5) use of prednisone for asthma exacerbation	Asthma medication use	192 exacerbations in 102 patients	6-11yo	RT-PCR	HRV, RSV, hMPV, ADV, INF, EV, CoV, PIV	69% of 192 exacerbations	HRV 34%	NR
Ducharme, 2016 - Canada (26)	cohort	ED	year round; 02/2011 to 12/2013	physician diagnosis of asthma, based on previous wheezing episode with signs of airflow obstruction and response to bronchodilators, ≥3 asthma-like episodes (if <2 years old), or previous diagnostic lung function tests	Clinical symptoms	965 (total study sample size 973)	1-17y, 75% 1-5y	RT-PCR	HRV-A, HRV-B, RSV, hMPV, ADV, INF, INF-A, INF-B, H1N1, H3N2, H5N1, PIV (types 1,2,3 and 4), CoV-HKU1, CoV-NL63, CoV-229E, CoV-OC43, Entero/rhinovirus, EV A, B, C, D	579/933 (62.1%)	HRV A, B or rhino/enterovirus - 54.2%	166/933 (17.8%)
Duenas 2016 - Colombia (42)	cross-sectional	ED	year round; 12/2010-03/2012	GINA-guidelines ;asthma diagnosis ≥6 months; by pediatrician/pulmonologist confirmed asthma	Clinical symptoms	169	2-15 y	RT-PCR ; DNA sequencing analysis; molecular typing	HRV, HRV-A, HRV-B, HRV-C, RSV, ADV, M pneumoniae, INF-A, INF-B, EV-D68, PIV types 1,2,3, Picornavirus	141/169 (83%)	HRV 125/169 (74%) - HRV-C 83 (49%)	21/169 dual M. pneumoniae and virus infection; 18/169 more than 1 viral pathogen
Hanhan, 2008 - USA (43)	cohort	hospitalized patients	year round; 09/1197 to 10/1998	status asthmaticus definition: failure to respond to usual appropriate initial emergency room treatment necessitating PICU admission	Clinical symptoms and asthma medication use	35	1.5-19y	serology	M pneumoniae	15/35 (42%)		
Jartti, 2007 - Finland (44)	RCT	hospitalized patients	year round; 09/2000 to 05/2002	NIH guidelines (2002)	Clinical symptoms	58	0.42-6.1 y, mean 2.6 yr (SD 1.3)	RT-PCR ; viral culture ; DFA testing on fresh specimen; serology	HRV, RSV, hMPV, ADV, INF-A, INF-B, PIV (types 1,2,3 and 4), EV, CoV	58158 (100% cf. presence of pathogen was inclusion criterium)	EV 21/58 (36%)	17/58 (29%)

Joao 2007 - Portugal (45)	cohort	ED	year round; 01/2003 to 12/2003	GINA-guidelines (2002)	GINA guidelines	37	6-13y (mean 8.5y)	RT-PCR ; IFA	HRV, RSV, ADV, INF, M pneumoniae, C pneumoniae, EV, PIV	78%	HRV 70.3%	(22%)
Kantor, 2016 - USA (46)	cohort	ED	year round; summer/2011 to spring/2015	history of physician-diagnosed asthma	Clinical symptoms, management of exacerbation and treatment reponse	155 (total study sample size 183)	6-17y, mean 9.9y (SD 3.2y)	RT-PCR ; Molecular typing	HRV, RSV, hMPV, ADV, INF-B, Entero/rhinovirus, PIV types 1,2,3, INF, INF-A types H1 and H3	123/183 (67%)	rhino/enterovirus 61% of 155	4/183 (2%)
Kato, 2011 - Japan (47)	cohort	hospitalized patients	year round; 11/2003 to 11/2006	history ≥3 episodes recurrent wheezing and documented wheezing by auscultation - based on Japanese Society of Pediatric Allergy and Clinical Immunology guidelines	Doctor diagnosis	33	0.3-8.1 y	RT-PCR ; antigen rapid testing ; DNA sequencing analysis	HRV, RSV, EV-D68, EV, coxackie/echovirus, PIV	33/33 (cf. inclusion pathogen positives - 100%)	HRV 21/33 (64%)	7/58 (12%) - analysis population: NR
Leung, 2010 - China (Hong Kong) (48)	cohort	hospitalized patients and outpatient clinic	year round; 01/2007-02/2008	hyperresponsiveness to methacholine or reversible airflow limitation or ≥ 3 episodes of cough, shortness of breath, and wheezing during previous 12 months, based on British Thoracic Society guideline/criteria	GINA guidelines	209	3-18y; mean 7.6 (SD 4.1)	RT-PCR	HRV, RSV, hMPV, ADV, HBoV, INFA (H1N1, H3N2, H5N1), INF-B, PIV (types 1,2,3 and 4), CoV-229E, CoV-OC43, SARS-CoV, EV, M pneumoniae, C pneumoniae	105/206 (51.0%)	26.2% (54/206)	22/206 (10.7%)
Maffey, 2010 - Argentina (49)	cohort	hospitalized patients	year round; 1/2006 to 12/2006	GINA-guidelines with history of ≥?previous wheezing episodes diagnosed by a physician, presenting with a new episode requiering hospitalization	not defined	137 (total study sample size 209)	0.4-16y	RT-PCR ; IFA	HRV, RSV, hMPV, ADV, bocavirus, INF A, INF B, CoV, Echovirus, ParaINF 1,2,3, Mycoplasma pneumoniae, Chlamydia pneumoniae	162/209 (78%)	RSV 85/209 (40.7%)	47/209 (22.5%) - 43/209 dual, 4/209 triple infection
Mak, 2011 - China (Hong Kong) (50)	case-control	hospitalized patients	fall and winter; 10/2008 to 03/2009	discharge diagnosis of asthma in Hospital computerized database	GINA guidelines	126	Mean 5.6y (SD 3.6y)	RT-PCR ; DNA sequencing analysis	HRV-A, HRV-B, HRV-C	NR - only reported for HRV	HRV 107/126 (85%) - HRV-C: 88/126 (70%)	NR
Malka, 2015 - USA (51)	cross-sectional	outpatient clinic	year round; 06/2010 to 05/2011	not defined	not defined	66	7-18y, mean 11y (SD 3.2)	RT-PCR	RSV, MPV, ADV, HBoV, INF-A, INF-B, PIV (types 1,2,3 and 4), Entero/rhinovirus, CoV, coxackie/echovirus	39/66 (59%)	HRV (62%)	11/39 (28%)
Mandelcwajg, 2010 - France (52)	cohort	ED	fall, winter; 11/2005 to 03/2009	previous doctor diagnosis of asthma or history ≥1 acute asthma episode	not defined	339	1.5-8.92y	RT-PCR ; viral culture ; DFA testing on fresh specimen	RSV, hMPV, ADV, HBoV, INF-A, INF-B, PIV types 1,2,3	123/339 (36.3%)	RSV 50/339 (14.8%)	NR
Nagayama, 1999 - Japan (53)	cohort	hospitalized patients	not reported	≥2 episodes of wheezy distress	Clinical symptoms and health service use	212 (number of exacerbation episodes)	0.5-14y	Bacterial culture	Haemophilus influenzae, Streptococcus pneumoniae	43/212 (20.3%)	H. influenzae 17/212 episodes (8.0%)	5/212 (2.4%)
Ou, 2008 - Taiwan (55)	cohort	ED and outpatient clinic	year round; 01/2000-12/2005	history ≥1 clinically evident asthma attacks after age 2 years	clinical symptoms and asthma medication use	316	2-14y	serology	M pneumoniae	99/316 (31.3%)		
Ozcan, 2011 - Turkey (54)	cohort	outpatient clinic	year round; 09/2009-09/2010	GINA-guidelines (2008)	GINA guidelines	104	mean 8.78 y (SD 3.4)	RT-PCR	HRV, RSV, hMPV, ADV, INF-A, INF-B, PIV (types 1,2,3 and 4), CoV-OC49, CoV-NL63, CoV-229E	56/104 (54%)	HRV 37/104 (36%)	9/104 (9%)
Rueter, 2012 - Australia (56)	cohort	ED	year round; 07/2002 to 09/2004	physician diagnosis of asthma attack based on clinical symptoms on presentation	Doctor diagnosis	135 (total study sample size 168)	2-16y, mean 6.6y (SD 3.5)	RT-PCR ; viral culture ; DFA testing on fresh specimen	HRV, RSV, hMPV, ADV, INF-A, INF-B, PIV	all investigated patients: 64% (107/168) - NR for 135 included	NR	NR
Smith, 2001 - USA (57)	case-control	ED	year round; 07/1996 to 05/1997	medical history of at least mild intermittent asthma	Clinical symptoms	101	3-18y	viral culture ; DFA fresh specimen; IFA	RSV, ADV, INF-A, INF-B, PIV 1,2,3	15/101 (15%)	RSV 7/101 (7%)	0 (0%)
Thumerelle, 2003 - France (58)	cohort	hospitalized patients	fall, winter, spring; 10/1998 to 06/1999	≥3 recurrent episodes of reversible wheezing within 2 years preceding study	Guidelines	82	2.1-15.33y, mean: 7.75y (SD 4.8y)	RT-PCR ; IFA ; serology	HRV, RSV, ADV, INF-A, INF-B, CoV-229E, EV, PIV types 1,2,3, M pneumoniae, C pneumoniae	37/82 (45%)	EV 13/82 (16%)	6/82 (7%)
Zhao, 2002 - Japan (59)	cohort	outpatient clinic	fall, winter; 10/1999 to 03/2000	NIH guidelines (1997)	not defined	28 (total study sample size 64)	0.33-15y, mean 4.14 (SD 3.55y)	Antigen rapid testing	RSV, ADV, INF-A	44% (28/64)	RSV 17/64 (27%)	0/64 (0%)

‡ED: Emergency Department †ADV: adenovirus, CoV: coronavirus, C. pneumoniae: Chlamydia pneumoniae, EV: enterovirus; H1N1: influenza virus Hemagglutinin Type 1 and Neuraminidase Type 1, HBoV: bocavirus, H. influenzae: Haemophilus influenzae, hMPV: human metapneumo virus, HRV: human rhinovirus, HRV-A: human rhinovirus A, INF: influenza virus, INF-A: influenza A virus, INF-B: influenza B virus, M. pneumoniae: Mycoplasma pneumoniae, PIV: parainfluenza virus

Appendix 1: Electronic Search Strategies

Search strategy: PubMed/MEDLINE – 1946 to October 19, 2016

#5,"Search (#1 AND #2 AND #3 AND #4)" (without any restrictions)

#4,"Search (""patient care""[Mesh] OR ""length of stay""[tw] OR hospitalization*[tw] OR hospitalisation*[tw] OR admission*[tw] OR emergency[tw] OR ""emergency""[Journal] OR ed[tiab] OR er[tiab] OR ""intensive care units""[Mesh] OR ""intensive care""[tw] OR ICU[tw] OR return visit*[tw] OR readmi*[tw] OR revisit*[tw] OR recurr*[tw] OR flare up*[tw] OR exacer*[tw] OR ""treatment outcome""[Mesh] OR treatment failure*[tw] OR outcome*[tw] OR disease progress*[tw] OR ""disease progression""[Mesh] OR ""Respiratory function tests""[Mesh] OR ""lung function"" OR FEV[tw] OR ""respiratory function""[tw] OR spirometry[tw] OR function test*[tw] OR pulmonary function*[tw] OR ventil*[tw] OR mortality[tw] OR morbidity[tw] OR fatal*[tw])"

#3,"Search (((""respiratory""[ti] AND infection*[tw]) OR (""respiratory""[tw] AND infection*[ti]) OR respiratory infection*[tw] OR ""Respiratory Tract Infections""[Mesh] OR Respiratory Tract Infection*[tw] OR virus[tw] OR viruses[tw] OR viral[tw] OR virology[subheading] OR virology[tw] OR ""virus diseases""[Mesh] OR ""adenoviruses, Human""[Mesh] OR adenovir*[tw] OR ""Influenza, Human""[Mesh] OR ""Influenza A virus""[Mesh] OR ""Influenza B virus""[Mesh] OR influenza*[tw] OR grippe[tw] OR flu[tw] OR RSV[tw] OR ""Respiratory Syncytial Virus, Human""[Mesh] OR ""Respiratory Syncytial Virus""[tw] OR respirovirus[Mesh] OR rubulavirus[Mesh] OR parainfluenza*[tw] OR metapneumovirus[Mesh] OR metapneumovirus*[tw] OR rhinovirus[Mesh] OR rhinovirus*[tw] OR enterovirus[Mesh] OR enterovirus*[tw] OR Coronavirus[Mesh] OR coronavirus*[tw] OR bocavirus[Mesh] OR bocavirus*[tw] OR bacteria*[tw] OR bacterium[tw] OR microbiology[sh] OR microbiology[tw] OR bacterial infections[mesh] OR Mycoplasma[Mesh] OR ""Mycoplasma infections""[Mesh] OR Mycoplasma[tw] OR ""Chlamydophila pneumoniae""[Mesh] OR Chlamydia*[tw] OR ""Whooping cough""[Mesh] OR ""Whooping Cough""[tw] OR Pertussis[tw] OR Bordetella[Mesh] OR bordetella[tw] OR ""Mycobacterium tuberculosis""[Mesh] OR tuberculosis[Mesh] OR tuberculosis[tw] OR ""streptococcus pneumoniae""[Mesh] OR streptococ*[tw] OR ""staphylococcus aureus""[Mesh] OR staphylococ*[tw])"

#2,"Search (Infan* OR newborn* OR new-born* OR perinat* OR neonat* OR baby OR baby* OR babies OR toddler* OR minors OR minors* OR boy OR boys OR boyfriend OR boyhood OR girl* OR kid OR kids OR child OR child* OR children* OR schoolchild* OR schoolchild OR school child[tiab] OR school child*[tiab] OR adolescen* OR juvenil* OR youth* OR teen* OR under*age* OR pubescen* OR pediatrics[mh] OR pediatric* OR paediatric* OR peadiatric* OR school[tiab] OR school*[tiab] OR prematur* OR preterm*)"

#1,"Search (Asthma[Mesh:noexp] OR asthma[tw] OR asthmatic*[All Fields])"

Search strategy: EMBASE Classic + EMBASE (platform: Ovid) – from 1947 to October 19, 2016

1 asthma/ or asthma*.tw.

2 (Infan* or newborn* or new-born* or perinat* or neonat* or baby or baby* or babies or toddler* or minors* or boy or boys or boyfriend or boyhood or girl* or kid or kids or child* or schoolchild* or adolescen* or juvenil* or youth* or teen* or under age* or underage* or pubescen* or pediatric* or paediatric* or peadiatric* or prematur* or preterm*).mp. or school*.ti,ab. or exp pediatrics/

3 ("respiratory".ti. and infection*.mp.) or ("respiratory".mp. and infection*.ti.) or respiratory infection*.mp. or Respiratory Tract Infection*.mp. or virus.mp. or viruses.mp. or viral.mp. or virology.mp. or adenovir*.mp. or influenza*.mp. or grippe.mp. or flu.mp. or RSV.mp. or "Respiratory Syncytial Virus".mp. or exp respirovirus/ or exp rubulavirus/ or parainfluenza*.mp. or metapneumovirus*.mp. or rhinovirus*.mp. or enterovirus*.mp. or Coronavirus*.mp. or bocavirus*.mp. or bacteria*.mp. or bacterium.mp. or microbiology.mp. or Mycoplasma.mp. or Chlamydia*.mp. or "Whooping Cough".mp. or Pertussis.mp. or bordetella.mp. or tuberculosis.mp. or streptococ*.mp. or staphylococ*.mp.

4 ("patient care" or "length of stay" or hospitali?ation* or admission* or emergency or ed or er or "intensive care" or ICU or return visit* or readmi* or revisit* or recurr* or flare up* or exacer* or treatment failure* or outcome* or disease progress* or "lung function" or FEV or "respiratory function" or spirometry or function test* or pulmonary function* or ventil* or mortality or morbidity or fatal*).mp. or emergency.jx.

5 1 and 2 and 3 and 4

Search strategy: BIOSIS Previews – from 1969 to October 19, 2016 and BIOSIS Previews Archive 1926 to 1968

1 asthma*.mp.
2 (Infan* or newborn* or new-born* or perinat* or neonat* or baby or baby* or babies or toddler* or minors* or boy or boys or boyfriend or boyhood or girl* or kid or kids or child* or schoolchild* or adolescen* or juvenil* or youth* or teen* or under age* or underage* or pubescen* or pediatric* or paediatric* or peadiatric* or prematur* or preterm*).mp. or school*.ti,ab.
3 ("respiratory".ti. and infection*.mp.) or ("respiratory".mp. and infection*.ti.) or respiratory infection*.mp. or Respiratory Tract Infection*.mp. or virus.mp. or viruses.mp. or viral.mp. or virology.mp. or adenovir*.mp. or influenza*.mp. or grippe.mp. or flu.mp. or RSV.mp. or "Respiratory Syncytial Virus".mp. or parainfluenza*.mp. or metapneumovirus*.mp. or rhinovirus*.mp. or enterovirus*.mp. or Coronavirus*.mp. or bocavirus*.mp. or bacteria*.mp. or bacterium.mp. or microbiology.mp. or Mycoplasma.mp. or Chlamydia*.mp. or "Whooping Cough".mp. or Pertussis.mp. or bordetella.mp. or tuberculosis.mp. or streptococc*.mp. or staphylococc*.mp.
4 ("patient care" or "length of stay" or hospitali?ation* or admission* or emergency or ed or er or "intensive care" or ICU or return visit* or readmi* or revisit* or recurr* or flare up* or exacer* or treatment failure* or outcome* or disease progress* or "lung function" or FEV or "respiratory function" or spirometry or function test* or pulmonary function* or ventil* or mortality or morbidity or fatal*).mp. or emergency.jx.
5 1 and 2 and 3 and 4

Search strategy: Cochrane Central Register of Controlled Trials (CENTRAL) – From inception to October 19, 2016

#1 MeSH descriptor: [Asthma] explode all trees
#2 asthma*:ti,ab,kw
#3 #1 or #2
#4 (Infan* or newborn* or new next born* or perinat* or neonat* or baby or baby* or babies or toddler* or minors* or boy or boys or boyfriend or boyhood or girl* or kid or kids or child* or schoolchild* or adolescen* or juvenil* or youth* or teen* or under next age* or underage* or pubescen* or pediatric* or paediatric* or peadiatric* or prematur* or preterm*):ti,ab,kw or school*:ti,ab
#5 MeSH descriptor: [Pediatrics] explode all trees
#6 #4 or #5
#7 "respiratory":ti and infection*
#8 "respiratory" and infection*:ti
#9 Respiratory next infection*:ti,ab,kw or Respiratory next Tract next Infection*:ti,ab,kw or virus:ti,ab,kw or viruses:ti,ab,kw or viral:ti,ab,kw or virology:ti,ab,kw or adenovir*:ti,ab,kw or influenza*:ti,ab,kw or grippe:ti,ab,kw or flu:ti,ab,kw or RSV:ti,ab,kw or "Respiratory Syncytial Virus":ti,ab,kw or parainfluenza*:ti,ab,kw or metapneumovirus*:ti,ab,kw or rhinovirus*:ti,ab,kw or enterovirus*:ti,ab,kw or Coronavirus*:ti,ab,kw or bocavirus*:ti,ab,kw or bacteria*:ti,ab,kw or bacterium:ti,ab,kw or microbiology:ti,ab,kw or Mycoplasma:ti,ab,kw or Chlamydia*:ti,ab,kw or "Whooping Cough":ti,ab,kw or Pertussis:ti,ab,kw or bordetella:ti,ab,kw or tuberculosis:ti,ab,kw or streptococc*:ti,ab,kw or staphylococc*:ti,ab,kw
#10 MeSH descriptor: [Respirovirus] explode all trees
#11 MeSH descriptor: [Rubulavirus] explode all trees
#12 #7 or #8 or #9 or #10 or #11
#13 ("patient care" or "length of stay" or hospitali?ation* or admission* or emergency or ed or er or "intensive care" or ICU or return next visit* or readmi* or revisit* or recurr* or flare next up* or exacer* or treatment next failure* or outcome* or disease next progress* or "lung function" or FEV or "respiratory function" or spirometry or function next test* or pulmonary next function* or ventil* or mortality or morbidity or fatal*) or emergency:so
#14 #3 and #6 and #12 and #13

Appendix 2. ROBINS-I Risk of bias assessment by exposure-outcome association

Study primary author, year, outcome	Confounding	Selection Bias	Exposure classification Bias	Bias due to Missing Data	Outcome Measurement Bias	Reporting Bias	Overall Bias
Severity of Asthma Exacerbation							
Akturk - 2016 - Severity scale: mild-Moderate-severe	Serious	Serious	Low	Moderate	Moderate	Moderate	Serious
Amin - 2013 - Hypoxia	Critical	Critical	Moderate	No information	Moderate	Serious	Critical
Amin - 2013 - Duration of oxygen therapy	Critical	Critical	Moderate	No information	Serious	Serious	Critical
Arden - 2010 - Severity scale: mild-Moderate-severe	Serious	Moderate	Low	Serious	Serious	Serious	Serious
Awasthi - 2012 - Severity scale: mild-Moderate-severe	Serious	Serious	Serious	No information	Low	Serious	Serious
Bebear - 2014 - Duration of oxygen therapy	Serious	Moderate	Moderate	No information	Moderate	Moderate	Serious
Berkovich - 1970 - Severity scale: mild-Moderate-severe	Critical	Low	Moderate	No information	Moderate	Moderate	Critical
Bizzintino - 2011 - Severity scale: mild-Moderate-severe (HRVC+ vs HRVA+ or HRVB+)	Moderate	Moderate	Low	Low	Low	Moderate	Moderate
Bizzintino - 2011 - Severity scale: mild-Moderate-severe (HRVC+ vs viral infection+ non-HRVC)	Moderate	Moderate	Low	Low	Low	Moderate	Moderate
Bizzintino - 2011 - Severity scale: mild-Moderate-severe (HRVC+ vs HRVC-)	Moderate	Moderate	Low	Low	Low	Moderate	Moderate
Coleman - 2015 - Severity scale: mild-Moderate-severe	Serious	Moderate	Low	Serious	No information	No information	Serious
Duenas 2016 - Severity scale: mild-Moderate-severe	Serious	Serious	Low	Moderate	Serious	Serious	Serious
Hanhan - 2008 - Duration of oxygen therapy	Serious	Serious	Moderate	Moderate	No information	Moderate	Serious
Joao 2007 - Severity scale: mild-Moderate-severe	Serious	Moderate	Moderate	Low	Moderate	Moderate	Serious
Kantor - 2016 - Hypoxia and oxygen therapy need	Serious	Serious	Low	No information	Moderate	Serious	Serious
Kantor - 2016 - Severity scale: mild-Moderate-severe	Serious	Serious	Low	No information	Moderate	Serious	Serious
Kato - 2011 - Severity scale: mild-Moderate-severe	Serious	Moderate	Low	No information	Moderate	Moderate	Serious
Leung - 2010 - Hypoxia and oxygen therapy need	Serious	Moderate	Low	Low	Moderate	Moderate	Serious
Maffey - 2010 - Duration of oxygen therapy	Critical	Moderate	Low	No information	Moderate	Serious	Critical
Mak - 2011 - Hypoxia and oxygen therapy need	Critical	Moderate	Low	Low	Low	Moderate	Critical
Ozcan - 2011 - Severity scale: mild-Moderate-severe	Serious	Serious	Low	No information	Moderate	Moderate	Serious
Thumerelle - 2003 - Duration of oxygen therapy (pathogen pos vs neg)	Serious	Moderate	Moderate	Moderate	Moderate	Moderate	Serious
Thumerelle - 2003 - Duration of oxygen therapy (viral pathogen + vs atypical bact +)	Serious	Moderate	Moderate	Moderate	Moderate	Moderate	Serious
Thumerelle - 2003 - Severity scale: mild-Moderate-severe (pathogen pos vs neg)	Serious	Serious	Moderate	Moderate	Moderate	Moderate	Serious
Thumerelle - 2003 - Severity scale: mild-Moderate-severe (viral pathogen + vs atypical bact +)	Serious	Serious	Moderate	Moderate	Serious	Moderate	Serious
Zhao - 2002 - Severity scale: mild-Moderate-severe	Critical	Serious	Serious	No information	Moderate	Moderate	Critical
Health Care Utilization							
Akturk - 2016 - Hospital admission	Serious	Serious	Low	Moderate	Moderate	Moderate	Serious
Akturk - 2016 - ICU admission	Serious	Serious	Low	Moderate	Moderate	Moderate	Serious
Amin - 2013 - ICU admission	Critical	Critical	Moderate	No information	Serious	Serious	Critical
Amin - 2013 - Need for systemic treatment	Critical	Critical	Moderate	No information	Serious	Serious	Critical
Awasthi - 2012 - Duration of hospitalization	Serious	Serious	Serious	No information	Low	Serious	Serious
Bebear - 2014 - Duration of hospitalization	Serious	Moderate	Moderate	No information	Moderate	Moderate	Serious
Belessis - 2004 - ICU admission	Critical	Serious	Serious	Serious	Moderate	Moderate	Critical
Ducharme - 2016 - Hospital admission	Serious	Moderate	Low	Moderate	Low	Moderate	Serious
Ducharme - 2016 - Relapse	Serious	Moderate	Low	Moderate	Low	Moderate	Serious
Duenas 2016 - Duration of hospitalization	Serious	Serious	Low	Serious	Serious	Serious	Serious
Duenas 2016 - Hospital admission	Serious	Serious	Low	Moderate	Serious	Serious	Serious
Duenas 2016 - ICU admission	Serious	Serious	Low	Moderate	Serious	Serious	Serious
Duenas 2016 - Mechanical ventilation	Serious	Serious	Low	Moderate	Serious	Serious	Serious
Duenas 2016 - Relapse	Serious	Serious	Low	Moderate	Serious	Serious	Serious
Hanhan - 2008 - Duration of hospitalization	Serious	Serious	Moderate	Moderate	No information	Moderate	Serious
Hanhan - 2008 - ICU admissionduration of	Serious	Serious	Moderate	Moderate	No information	Moderate	Serious
Jartti - 2007 - Duration of hospitalization	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Jartti - 2007 - Duration of hospitalization (picornavirus+ vs picornavirus-)	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Jartti - 2007 - Duration of hospitalization 2 (EV+ vs EV-)	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Jartti - 2007 - Relapse outpatient visit (HRV+ vs HRV-)	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Jartti - 2007 - Rehospitalization (HRV+ vs HRV-)	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Jartti - 2007 - Relapse outpatient visit (EV+ vs EV-)	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Jartti - 2007 - Rehospitalization (EV+ vs EV-)	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Kantor - 2016 - Duration of hospitalization	Serious	Serious	Low	No information	Moderate	Serious	Serious

Kantor - 2016 - Hospital admission	Serious	Serious	Low	No information	Moderate	Serious	Serious
Kantor - 2016 - ICU admission	Serious	Serious	Low	No information	Moderate	Serious	Serious
Kato - 2011 - Duration of hospitalization	Serious	Moderate	Low	No information	Moderate	Moderate	Serious
Leung - 2010 - Duration of hospitalization	Serious	Moderate	Low	Low	Serious	Moderate	Serious
Maffey - 2010 - Duration of hospitalization	Critical	Moderate	Low	No information	Moderate	Serious	Critical
Mak - 2011 - Duration of hospitalization	Critical	Moderate	Low	Low	Low	Moderate	Critical
Mak - 2011 - ICU admission	Critical	Moderate	Low	Low	Low	Moderate	Critical
Mandelcwajg - 2010 - Hospital admission (INF)	Critical	Moderate	Moderate	Low	No information	Low	Critical
Mandelcwajg - 2010 - Hospital admission (virus+, non-INF)	Critical	Moderate	Moderate	Low	No information	Low	Critical
Ou - 2008 - Relapse	Serious	Moderate	Serious	Low	Serious	Moderate	Serious
Ozcan - 2011 - Hospital admission	Serious	Serious	Low	No information	Moderate	Moderate	Serious
Smith - 2001 - ICU admission	Serious	Serious	Moderate	Low	Moderate	Moderate	Serious
Thumerelle - 2003 - Duration of hospitalization (pathogen pos vs neg)	Serious	Moderate	Moderate	Moderate	Serious	Moderate	Serious
Thumerelle - 2003 - Duration of hospitalization (viral pathogen + vs atypical bact +)	Serious	Moderate	Moderate	Moderate	Serious	Moderate	Serious
Response to Treatment							
Ducharme - 2016 - Duration of symptoms after treatment (time PRAM-score ≥4)	Moderate	Moderate	Low	Moderate	Low	Moderate	Moderate
Ducharme - 2016 - Duration of symptoms after treatment (time to PRAM<=3)	Moderate	Moderate	Low	Moderate	Low	Moderate	Moderate
Ducharme - 2016 - Duration of symptoms after treatment (10 day resolution)	Moderate	Moderate	Low	Serious	Low	Moderate	Serious
Ducharme - 2016 - length of active treatment	Moderate	Moderate	Low	Moderate	Low	Moderate	Moderate
Ducharme - 2016 - number of doses bronchodilatators needed	Serious	Moderate	Low	Moderate	Low	Moderate	Serious
Ducharme - 2016 - treatment failure yes/no	Moderate	Moderate	Low	Moderate	Low	Moderate	Moderate
Ducharme - 2016 - treatment failure yes/no	Serious	Moderate	Low	Moderate	Low	Moderate	Serious
Duenas 2016 - Need of steroids yes/no	Serious	Serious	Low	Moderate	Serious	Serious	Serious
Hanhan - 2008 - number of doses bronchodilatators needed	Serious	Serious	Moderate	Moderate	No information	Moderate	Serious
Kantor - 2016 - length of active treatment (composite score)	Moderate	Serious	Low	No information	Moderate	Serious	Serious
Kantor - 2016 - number of hours beta-agonist weaning	Serious	Serious	Low	No information	Moderate	Serious	Serious
Kato - 2011 - length of active treatment	Serious	Moderate	Low	No information	Moderate	Moderate	Serious
Malka - 2015 - change in FEV1 folLowing albuterol	Serious	Critical	Low	Moderate	Low	Serious	Critical
Malka - 2015 - FEV1	Serious	Critical	Low	Moderate	Low	Serious	Critical
Malka - 2015 - length of active treatment	Serious	Critical	Low	Moderate	Low	Serious	Critical
Ozcan - 2011 - Need of steroids yes/no	Serious	Serious	Low	No information	Moderate	Moderate	Serious
Rueter - 2012 - number of doses bronchodilatators needed (pathogen+ vs pathogen-)	Serious	Moderate	Low	Serious	Moderate	Moderate	Serious
Rueter - 2012 - number of doses bronchodilatators needed HRV+ vs HRV-)	Serious	Moderate	Low	Moderate	Moderate	Moderate	Serious
Rueter - 2012 - number of doses bronchodilatators needed	Serious	Moderate	Low	Moderate	Moderate	Moderate	Serious
Morbidity and Mortality							
Akturk - 2016 - consolidation/pneumonia	Serious	Serious	Low	Moderate	Moderate	Moderate	Serious
Akturk - 2016 - Duration of symptoms	Serious	Serious	Low	Moderate	Moderate	Moderate	Serious
Amin - 2013 - consolidation	Critical	Critical	Moderate	No information	Serious	Serious	Critical
Amin - 2013 - infiltration	Critical	Critical	Moderate	No information	Serious	Serious	Critical
Amin - 2013 - death	Critical	Critical	Moderate	No information	Low	Serious	Critical
Arden - 2010 - Cough score	Serious	Moderate	Low	Serious	Serious	Serious	Serious
Arden - 2010 - Quality of life (coinfection vs single pathogen)	Serious	Moderate	Low	Serious	Serious	Serious	Serious
Arden - 2010 - Quality of life (HRVA vs HRVC)	Serious	Moderate	Low	Serious	Serious	Serious	Serious
Awasthi - 2012 - consolidation/pneumonia	Serious	Serious	Serious	No information	Moderate	Serious	Serious
Bebear - 2014 - consolidation/pneumonia	Serious	Serious	Serious	No information	Serious	Moderate	Serious
Ducharme - 2016 - Quality of life	Moderate	Moderate	Low	Serious	Low	Moderate	Serious
Duenas 2016 - consolidation/pneumonia	Serious	Serious	Low	Moderate	Serious	Serious	Serious
Hanhan - 2008 - consolidation/pneumonia	Serious	Serious	Moderate	Moderate	Moderate	Moderate	Serious
Jartti - 2007 - Duration of symptoms	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Jartti - 2007 - Duration of symptoms	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Jartti - 2007 - Duration of symptoms	Serious	Moderate	Low	Moderate	Low	Serious	Serious
Kantor - 2016 - PEF % pred	Serious	Serious	Low	No information	Moderate	Serious	Serious
Kato - 2011 - duration of wheezing	Serious	Moderate	Low	No information	Serious	Moderate	Serious
Leung - 2010 - Duration of symptoms	Serious	Moderate	Low	Low	Moderate	Moderate	Serious
Leung - 2010 - FEV1	Serious	Moderate	Low	Low	Moderate	Moderate	Serious
Nagayama - 1999 - consolidation/pneumonia	Critical	Serious	Low	Low	Moderate	Low	Serious
Ou - 2008 - consolidation/pneumonia	Serious	Moderate	Serious	Low	Moderate	Moderate	Serious

Ozcan - 2011 - Duration of symptoms	Serious	Serious	Low	No information	Serious	Moderate	Serious
Thumerelle - 2003 - consolidation/pneumonia (pathogen pos vs neg)	Serious	Serious	Moderate	Moderate	Moderate	Moderate	Serious
Thumerelle - 2003 - consolidation/pneumonia (viral pathogen + vs atypical bact +)	Serious	Serious	Moderate	Moderate	Serious	Moderate	Serious
Thumerelle - 2003 - Improvement at 48h (pathogen pos vs neg)	Serious	Moderate	Moderate	Moderate	Serious	Moderate	Serious
Thumerelle - 2003 - Complete recovery (pathogen pos vs neg)	Serious	Moderate	Moderate	Moderate	Serious	Moderate	Serious
Thumerelle - 2003 - Improvement at 48h (viral pathogen + vs atypical bact +)	Serious	Moderate	Moderate	Moderate	Serious	Moderate	Serious
Thumerelle - 2003 - Complete recovery (viral pathogen + vs atypical bact +)	Serious	Moderate	Moderate	Moderate	Serious	Moderate	Serious

Low: study is comparable to a well-performed randomized trial with regard to this domain,
Moderate: study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well- performed randomized trial,
Serious: study has some important problems,
Critical: study is too problematic to provide any useful evidence on the effects of intervention,
No information on which to base a judgement about risk of bias for this domain

Appendix 3. Results organized by outcome domain

Study primary author, year	Number of patients	Exposure	Comparator	Outcome	Effet measure	Statistical method	Outcome numbers	Crude EM	Adjustment and interaction	Overal Risk of bias
Severity of Asthma Exacerbation										
Akturk, 2016	125 - Entire population	Pathogen positive	Pathogen negative	Asthma exacerbation severity: mild, moderate, severe- GINA-guidelines (2015)	presentation of proportions	Inference testing: X-square test, X-square with Yates' correction and Fisher's exact test	mild, moderate, severe in exposed: 48 (45%), 22 (21%), 36 (34%) vs in non-exposed 15 (79%), 4 (21%) severe 0	(p-value=0.006)	_	Serious
Amin, 2013	130 - Entire population	Pathogen positive	Pathogen negative	Hypoxia	presentation of proportions	Inference testing: Fisher's exact test	exposed 92.6% (50/54) vs non-exposed 84.2% (64/76)	(p-value: 0.392)	_	Critical
Amin, 2013	130 - Entire population	Pathogen positive	Pathogen negative	Duration of oxygen therapy (in days)	presentation of means	Inference testing: Fisher's exact test	exposed 4.1 (SD 2.4) vs non-exposed 5.2 (SD 5.5)	(p-value: 0.583)	_	Critical
Arden, 2010	39 (23 vs 16)- Subpopulation	HRV-A positive	HRV-C positive	Asthma symptom scores on day 28 post exacerbation presentation	presentation of median	Inference testing: Kruskal– Wallis test	HRV-A exposed 0.8 (IQR 0 – 1.5) vs HRV-C exposed 0	(p-value=0.01)	_	Serious
Awasthi, 2012	44 - Entire population	C. pneumoniae positive	C. pneumoniae negative	moderate exacerbation, severe exacerbation, imminent respiratory faillure - GINA 2009	presentation of proportions; OR	Inference testing: X-square and Fisher-exact	exposed 2/11 (18.2 %) moderate exacerbation, 9/11 (81.8 %) severe exacerbation vs non-exposed 14/33 (42.4%) moderate exacerbation, 17/33 (51.5%) severe exacerbation, 2/33 (6.1%) imminent respiratory faillure	OR for C. pneumoniae positivity in patients with severe exacerbation: 3.71, 95 % CI 0.58-29.77, (p-value=0.11; X-squared= 2.51)	_	Serious
Bebear, 2014	168 - Entire population	M. pneumoniae positive	M. pneumoniae negative	Duration of oxygen therapy (in days)	presentation of means	univariate and multivariate logistic regression	exposed 0 (CI 0;2) vs.non-exposde 0 (CI 0;1)	reported as statistical non-significant differences	model not provided - reported as statistical non-significant differences	Serious
Berkovich, 1970	79 - Entire population	Pathogen positive	Pathogen negative	Severity of respiratory distress symptoms	presentation of proportions	_	Pathogen positives mild, moderate, severe disease: 10/33 (30%), 14/33 (42%), 9/33 (27%) vs pathogen negatives mild, modereate, severe disease: 33/75 (44%), 31/75 (41%) , 11/75 (15%)	_	_	Critical
Bizzintino, 2011	110 (76 + 34)- Subpopulation	HRV-C positive	HRV-A or HRV-B positive	Acute Asthma Severity Score (modified NIH-score); on presentation to the hospital	presentation of means, confidence intervals	Inference testing: t-test; multivariate linear regression	HRV-C exposed 10.4 vs HRV-A/HRV-B exposed 9.5	HRV-C positive 95% CI 10.0-10.9 vs HRV-A/HRV-B positive 95% CI: 8.7 - 10.3 - (p-value=0.028)	Adjusted for age, sex. Linear regression coefficient not reported -(p-value=0.018); no interaction terms	Moderate
Bizzintino, 2011	128 (76 + 40)- Subpopulation	HRV-C positive	HRV-C negative and other viral pathogen positive	Acute Asthma Severity Score (modified NIH-score); on presentation	presentation of means, confidence intervals	Inference testing: t-test; multivariate linear regression	HRV-C exposed 10.4 vs viral pathogen non-HRV-C exposed 9.4	HRV-C exposed 95% CI 10.0–10.9 vs viral pathogen non-HRV-C exposed 95% CI 8.7–10.1 - (p-value=0.013)	Adjusted for age, sex. Linear regression coefficient not reported -(p-value=0.009); no interaction terms	Moderate
Bizzintino, 2011	126 - Sub population	HRV-C positive	HRV-C negative	Acute Asthma Severity Score (modified NIH-score); on presentation	presentation of means, confidence intervals	Inference testing: t-test; multivariate linear regression	HRV-C exposed 10.4 vs HRV-C non-exposed 9.4	HRV-C exposed 95% CI 10.0–10.9 vs HRV-C non-exposed 95% CI 8.7–10.2; (p-value=0.015)	Adjusted for age, sex. Linear regression coefficient not reported; (p-value=0.016); no interaction terms used	Moderate
Coleman, 2015	102 - Entire population	Pathogen positive	Pathogen negative	A severe asthma exacerbation was defined by the use of oral corticosteroids.	presentation of proportions	Inference testing	Severe in exposed: 14/60 (23%) vs non-exposed 23/132 (17%)	(P=0.34)	_	Serious
Duenas, 2016	169 - Entire population	M. pneumoniae and virus positive	pathogen negative	degree of severity of the exacerbation classified according to the pulmonary index	presentation of proportions	Inference testing: X-qsuare and Fisher's exact	M pneumoniae and viral pathogen exposed: Mild 8/21 (38.1%), Moderate 13/21 (61.9%), severe 0/21 (0%) vs non-exposed: Mild 11/25 (44.0%), Moderate 12/25 (48.0%) , Severe 2/25 (8.0%)	(p-value=0.705)	_	Serious
Hanhan, 2008	35 - Entire population	M. pneumoniae positive	M. pneumoniae negative	Duration of oxygen therapy (in days)	presentation of means/median	Inference testing: t-test or chi-square	exposed 3.5 vs non-exposed 3.35	(p-value =NS)	_	Serious
Joao, 2007	37 - Entire population	HRV positive vs Mycoplasma positive vs co-infection	Pathogen negative	Asthma exacerbation severity: mild-moderate-severe - GINA guidelines (2002)	presentation of proportions	Inference testing: X-qsuare	mild-moderate-severe: HRV exposed 10(50.0%), 7(50.0%),2(66.7%) vs Mycoplasma exposed: 2(10.0%), 0(0%), 0(0%) vs coinfection: 5(25%), 2(14.3%), 1(33.3) vs non-exposed: 3(15.0%), 5(35.7%), 0(0%)	(p-value>0.05)	_	Serious

		positive								
Kantor, 2016	155 - Subpopulation	HRV positive	Pathogen negative	Supplemental oxygen requirement yes/no	OR	univariate binomial logistic regression	_	OR 4.53 (95%CI 1.94-10.58), (p=0.001)	_	Serious
Kantor, 2016	155 - Subpopulation	HRV positive	Pathogen negative	Modified Pulmonary Index Score (MPIS): 6 categories (score 0-3): oxygen saturation, accessory muscle use, inspiratory to expiratory flow ratio, degree of wheezing, heart rate, and respiratory rate.	regression coefficients	univariate linear regression	_	Coeficient HRV exposed 2.76, (95%CI 1.66-3.87), (p-value<0.001)	_	Serious
Kato, 2011	33 (21+12)-Subpopulation	HRV positive	RSV positive	Symptom severity score: Mild (score 1); moderate (score 2); severe (score 3) (Japanese Society of Pediatric Allergy and Clinical Immunology)	presentation of means	Inference testing	HRV exposed 2.0 (SD 0.4) vs RSV exposed: 1.8 (SD 0.5)	_	_	Serious
Leung, 2010	209 - Entire population	HRV positive	HRV negative	minimum Sa02 (%)	presentation of means	Inference testing: t-test	exposed 93.8 (2.8) vs non-exposed 94.3 (2.3)	(p-value=0.333)	_	Serious
Maffey, 2010	137 - Subpopulation	RSV positve	HRV positive	Duration of oxygen therapy (in days)	presentation of means	Inference testing	RSV exposed 5.1 vs HRV exposed 3.4	(p=0.005)	_	Critical
Mak, 2011	126 - Subpopulation	HRV positive	HRV-A positive	Need for oxygen supplementation	presentation of proportions	Inference testing	HRV exposed 26/88 (29.5%) vs HRV-A exposed 6/19, (31.6%)	(p-value=0.920)	_	Critical
Ozcan, 2011	104 - Entire population	Pathogen positive	Pathogen negative	Asthma exacerbation severity: mild, moderate, severe - GINA-guidelines (2008)	presentation of proportions	Inference testing	Mild, Moderate, Severe in exposed: 5 (8.9%), 42 (75%), 9 (16.1%) vs in non-exposed 5 (10.4%), 31 (64.6%), 12 (25.0%)	(p-value=0 .477)	_	Serious
Thumerelle, 2003	82 - Entire population	Pathogen positive	Pathogen negative	Duration of oxygen therapy (in days)	presentation of means	Inference testing: t-test	exposed 0.93 (SD 1.8) vs non-exposed 0.68 (SD 0.95)	(p-value=0.481)	_	Serious
Thumerelle, 2003	37 (29+8) - Subpopulation	Viral pathogen only positive	Atypical bacteriae positive	Duration of oxygen therapy (in days)	presentation of means	Inference testing: Fisher exact and Wilcoxon test	virus exposed 0.6 (SD 1.1) vs atypical bacteriae exposed 2.1 (SD 3)	(p-value=0.153)	_	Serious
Thumerelle, 2003	82 - Entire population	Pathogen positive	Pathogen negative	Asthma exacerbation severity: mild-moderate-severe -international pediatric consensus statement on the management of childhood asthma (1998) and British Thoracic Society Guidelines (1993)	presentation of proportions	Inference testing: X-qsuare	exposed: mild 13%; moderate 51%, severe, 35% vs non-exposed: Mild 27%; moderate 41%; severe 29%	(p-value=0.287)	_	Serious
Thumerelle, 2003	37 (29+8) - Subpopulation	Viral pathogen positive only	Atypical bacteriae positive	Asthma exacerbation severity: mild-moderate-severe -international pediatric consensus statement on the management of childhood asthma (1998) and British Thoracic Society Guidelines (1993)	presentation of proportions	Inference testing: Fisher exact and Wilcoxon test	viral pathogen exposed: Mild, 13.8%; moderate, 58.5%; severe, 27.6% vs iatypical bacteriae exposed: Mild, 12.5%; moderate: 25%; severe, 62.5%	(p-value=0.138)	_	Serious
Zhao, 2002	28 - Subpopulation	RSV positive	INF-A positive	Asthma exacerbation severity: moderate or severe (lung function tests - saturation) - guidelines published NIH (1997) - proportion moderate or severe	presentation of proportions	Inference testing: X-square	RSV exposed 82% vs INF exposed 36%	(p-value= <0.05)	_	Critical
Health Care Utilization										
Akturk, 2016	125 - Entire population	Pathogen positive	Pathogen negative	Hospitalization yes/no	presentation of proportions	Inference testing: X-square test, X-square with Yates' correction and Fisher's exact test	exposed 32 (30.2%) vs non-exposed 1 (5.3)	(p-value=0.023)	_	Serious
Akturk, 2016	125 - Entire population	Pathogen positive	Pathogen negative	PICU admission yes/no	presentation of proportions	Inference testing: X-square test, X-	exposed 8 (7.7%) vs non exposed 0	(p-value=0.214)	_	Serious

						square with Yates' correction and Fisher's exact test				
Amin, 2013	130 - Entire population	Pathogen positive	Pathogen negative	ICU admission yes/no	presentation of proportions	Inference testing: Fisher's exact	exposed 22.2% (12/54) vs non-exposed 28.9% (22/76)	(p-value: 0.425)	_	Critical
Amin, 2013	130 - Entire population	Pathogen positive	Pathogen negative	Mechanical ventilation (no definition given)	presentation of proportions	Inference testing (X-qsuare, t-test, Fisher exact, Wilcoxon...)Fisher's exact	Mechanical ventilation: exposed 18.5% (10/54) vs non-exposed 21.1% (16/76)	(p-value=0.825)	_	Critical
Awasthi, 2012	44 - Entire population	C. pneumoniae positive	C. pneumoniae negative	Duration of hospitalization (in days)	presentation of means	Inference testing	exposed 9 (SD 2.19) vs non-exposed 7.19 (SD 2.10)	(p-value=0.02)	_	Serious
Bebear, 2014	168 - Entire population	M. pneumoniae positive	M. pneumoniae negative	Duration of hospitalization (in days)	presentation of means	univariate (and multivariate) logistic regression model	exposed 3 (CI 2-5) vs non-exposed 3 (CI 3-4)	reported as statistical non-significant differences	model not provided - reported as statistical non-significant differences	Serious
Belessis, 2004	92 - Entire population	Pathogen positive	Pathogen negative	ICU admission	presentation of proportions	Inference testing: X-qsuare	ICU-admitted patients: exposed 35/41 (85%) vs Non-ICU admitted: exposed to infection 44/51 (86%)	(p-value: reported to be non-significant)	_	Critical
Ducharme, 2016	965 - Entire population	Pathogen positive	Pathogen negative	Admission yes/no	presentation of proportions	_	exposed 95 (16.6%) vs non-exposed 42 (12.0%)	_	_	Serious
Ducharme, 2016	965 - Entire population	Pathogen positive	Pathogen negative	Relapse to emergency department within 7 days yes/no	presentation of proportions; OR	Logistic regression model	exposed 35 (6.2%) vs non-exposed 10 (2.9%)	OR 2.20 (95% 1.08, 4.51)	_	Serious
Duenas 2016	41 - Subpopulation	M pneumoniae and virus positive	Virus alone positive vs M. pneumoniae and virus negative	Hospitalization length (in days)	presentation of means	Inference testing	M pneumoniae and virus exposed 3.8 (SD 2.1) vs virus alone 3.2 (SD 1.9) vs non-exposed 2.7 (SD 1.2)	(p-value=0.741)	_	Serious
Duenas 2016	169 - Entire population	M pneumoniae and virus positive	Virus alone positive vs M. pneumoniae and virus negative	Need for hospitalization yes/no	presentation proportions	Inference testing	exposed 4 (19.0%) vs virus alone: 34/123 (27.6%) vs non-exposed 3 (12.0%)	(p-value=0.210)	_	Serious
Duenas 2016	169 - Entire population	M pneumoniae and virus positive	Virus alone positive vs M. pneumoniae and virus negative	ICU admission yes/no	presentation of proportions	Inference testing	exposed: 0 (0%) vs virus alone: 3/123 (2.4%) vs non-exposed 0 (0%)	(p-value=0.565)	_	Serious
Duenas 2016	169 - Entire population	M pneumoniae and virus positive	Virus alone positive vs M. pneumoniae and virus negative	Mechanical ventilation yes/no	presentation of proportions	Inference testing	exposed: 0 (0%) vs virus alone: 0/123 (0%) vs non-exposed 0 (0%)	(p-value=0)	_	Serious
Duenas 2016	169 - Entire population	M pneumoniae and virus positive	Virus alone positive vs M. pneumoniae and virus negative	Hospital readmissions	presentation of proportions	Inference testing	exposed 0 (0%) vs virus alone: 13/123 (10.6%) vs non-exposed 1 (4.0%)	(p-value=0.188)	_	Serious
Hanhan, 2008	35 - Entire population	M. pneumoniae positive	M. pneumoniae negative	Duration of hospitalization (in days)	presentation of means	Inference testing: X-qsuare, t-test	exposed 5.2 days vs non-exposed 4.65	(p-values =NS)	_	Serious
Hanhan, 2008	35 - Entire population	M pneumoniae positive	M pneumoniae negative	lenght of PICU stay (in days)	presentation of means	Inference testing: t-test or X-square	exposed 2.75 vs non-exposed 2.65	(P-values =NS)	_	Serious
Jartti, 2007	58 - Entire population	HRV positive	HRV negative	time until discharge (in hours)	presentation of median	multivariate linear regression with backward stepwise	HRV positive, steroids treated: 6 (IQR 6-30) vs HRV negative, steroids treated: 21 (IQR 6-42); HRV positive placebo 28 (IQR 18-54) vs HRV negative placebo 21 (IQR 6-39)	_	univariate analysis for effect prednisolone:interaction rhinovirus and steroids: p-value=0.05 ; multivariate analysis for effect prednisolone, interaction prednisolone and rhinovirus:p-value=0.37	Serious
Jartti, 2007	58 - Entire population	Picornavirus positive	Picornavirus negative	time until ready for discharge (in hours)	presentation of median	multivariate linear regression with backward stepwise	Picorna positive, steroids treated: 12 h, Picorna positive steorids non-treated : 24 hr, Picorna negative and steroids treated: 54 h, Picorna negative, steroids non-treated: 6 hr	_	Interaction picornavirus and prednisolone in univariate regression: p=0.026. Interaction picornavirus and prednisolone in multivariate regression: p-value=0.0022 with adjustment for study drug, picorna virus infection, RSV virus inection, study drug *picornavirus infection. In picornavirusgroup: RR 1.91, 95% CI 1.22-3.00, p=0.0048; in non-picornavirus group RR 0.30, 95%CI 0.11-0.82, p=0.019	Serious

Jartti, 2007	58 - Entire population	Enterovirus positive	Enterovirus negative	time until ready for discharge (in hours)	presentation of median	multivariate linear regression with backward stepwise	Enterovirus positive, steroids treated: 6 (IQR 6-18) vs in enterovirus negatives steroid treated: 30 (IQR 6-42); enterovirus positives non-treated: 35 (IQR 21-58) vs enterovirus negatives non-treated: 18 (IQR 6-30)	_	Univariate analysis - interaction enterovirus and prednisolone: p-value=0.0002. Multivariate analysis - interaction enterovirus and steroids: p-value=0.0007; In enterovirus group: RR 3.75, 95% CI 1.99–7.08, p < 0.0001. In non-enterovirus group, RR 0.84, 95% CI 0.50–1.39, p = 0.49. Adjustment for study drug, enterovirus infection, picornavirus infection, antibiotic treatment, study drug*enterovirus infection	Serious
Jartti, 2007	46 - Subpopulation	HRV positive	HRV negative	Outpatient visit as a result of wheezing during 2 months after discharge	presentation of proportions	multivariate linear regression with backward stepwise	HRV positive and steroids treated 1/5 (20%) vs HRV negative and steroid treated 2/16 (13%); HRV positive steroids non-treated 2/8 (25%) vs HRV negative non-treated 3/17 (18%)	_	univariate analysis: prednisolone vs placebo with interaction prednisolone and HRV: rhinovirus p=0.92; Multivariate analysis: interaction steroids and HRV: p-value=0.99	Serious
Jartti, 2007	46 - Subpopulation	Enterovirus positive	Enterovirus negative	Outpatient visit as a result of wheezing during 2 months after discharge	presentation of proportions	multivariate linear regression with backward stepwise	Number of outpatient visits in enterovirus pos, steroids treated: 2/9 (22%) vs in enterovirus neg 1/12 (8%). In enterovirus pos non-treated: 4/11 (36%) vs in enterovirus neg non-treated 1/14 (7%)	_	univariate analysis: prednisolone vs placebo with interaction prednisolone and HRV: rhinovirus p=0.79; Multivariate analysis: interaction steroids and HRV: p-value=0.49	Serious
Jartti, 2007	46 - Subpopulation	HRV positive	HRV negative	Number of rehospitalizations	presentation of proportions	multivariate linear regression with backward stepwise	HRV positive, steroid treated: 0/5 (0%) vs HRV neg treated 2/17 (12%); HRV positive non-treated: 1/8 (13%) vs HRV negaitve non-treated 2/17 (12%)	_	Interaction prednisolone and HRV: Univariate analysis: p-value=0.89; Multivariate analysis: p-value=0.97	Serious
Jartti, 2007	46 - Subpopulation	Enterovirus positive	Enterovirus negative	Number of rehospitalizations	presentation of proportions	multivariate linear regression with backward stepwise	enterovirus pos, steroids treated: 2/9 (22%) vs in enterovirus neg 0/12 (0%). In enterovirus pos non-treated: 2/11 (18%) vs in enterovirus neg non-treated 1/14 (7%)	_	Unvariate analysis, interaction enterovirus and prednisolone p-value=0.97; Multivariate analysis, interaction enterovirus and prednisolone p-value=0.97	Serious
Kantor, 2016	155 - Subpopulation	HRV +	Pathogen negative	Hospital length of stay	regression coeffcients	univariate linear regression	No numbers reported	exposed 21.43 (95%CI 10.53-32.32), (p-value<0.001)	_	Serious
Kantor, 2016	155 - Subpopulation	HRV positive	Pathogen negative	Hospital admission yes/no	OR	binomal logistic regression	No numbers reported	OR 2.92 (95%CI 1.49-5.73), (p-value=0.002)	_	Serious
Kantor, 2016	155 - Subpopulation	HRV positive	Pathogen negative	ICU admission yes/no	OR	binomal logistic regression	No numbers reported	OR 3.64, (95%CI 1.65-8.03), (p-value=0.001)	_	Serious
Kato, 2011	33 (21+12)-Subpopulation	HRV positive	RSV positive	Admission period (days)	presentation of means	Inference testing	HRV exposed 9.0 (SD 4.5) vs RSV exposed 7.7 (SD 2.3)	NR	_	Serious
Leung, 2010	209 - Entire population	HRV positive	HRV negative	Duration of hospitalization (in days)	presentation of means	Inference testing: t-test	exposed 3.4 (SD 1.4) vs non-exposed 3.7 (SD 2.0)	(p-value=0.349)	_	Serious
Maffey, 2010	137 - Subpopulation	RSV positive	HRV positive	Duration of hospitalization (in days)	presentation of means	Inference testing	RSV exposed 6.7 vs 5.2 in HRV exposed	(p-value=0.012)	_	Critical
Mak, 2011	107 (88+19) - Subpopulation	HRV-C positive	HRV-A positive	Duration of hospitalization (in days)	presentation of means	Inference testing	HRV-C positive 2.9 (SD 3.5) vs HRV-A positive 3.7 (SD 3.8)	(p-value=0.382)	_	Critical
Mak, 2011	126 - Subpopulation	HRV positive	HRV-A positive	Need for intensive care yes/no	presentation of proportions	Inference testing	HRV-C exposed 2/88 (2.3%) vs HRV-A exposed 0/19 (0%)	(p-value=1.000)	_	Critical
Mandelcwajg, 2010	339 - Entire population	Influenza positive in hospitalized patients	Influenza positive in ambulatory patients	Hospitalization yes/no	presentation of proportions	Inference testing: X--square and Fischer exact	Influenza A virus in hospitalized patients 6/232 (2.6%) vs Influenza A positive in ambulatory patients: 15/107 (14.1%)	(p-value <0.001)	_	Critical
Mandelcwajg, 2010	339 - Entire population	Pathogen positive in hospitalized patients	Pathogen positive in ambulatory patients	Hospitalization yes/no	presentation of proportions	Inference testing: X-square and Fischer exact	RSV hospitalized: 31/232 (13.8%) vs RSV ambulatory: 19/107 (17.7%); BoV hospitalized: 27/232 (11.6%) vs BoV ambulatory: 14/107 (13.1%); PIV-3 hospitalized:: 2/232 (0.8%) vs PIV-3 ambulatory : 1/107 (0.6%); hMPV positives: 5/61 (8.2%), ambulatory: 1/29 (2.5%); adenovirus hospitalized: 1/232 (0.3%), adenovirus ambulatory: 1/107 (0.6%)	NR	_	Critical
Ou, 2008	316 - Subpopulation	M. pneumoniae positive	M pneumoniae negative	Asthma recurrences one month after the first attack	presentation of proportions	Inference testing	exposed36/57 (62%) vs non-exposed 19/71 (27%)	(p-value=0.03)	_	Serious
Ozcan, 2011	104 - Entire population	Pathogen positive	Pathogen negative	Hospitalization yes/no (GINA guidelines)	presentation of proportions	Inference testing	exposed 11 (19.6%) vs non-exposed 6 (12.5%)	(p-value=0 .326)	_	Serious
Smith, 2001	101 - Entire population	RSV positive	RSV postitive	Admission to the PICU	presentation of proportions	Inference testing	ICU admitted patients exposed 3/11 vs discharged or ward admitted exposed 4/90.	(p=0.0269)	_	Serious
Thumerelle, 2003	82 - Entire population	Pathogen positive	Pathogen negative	Duration of hospitalization (in days)	presentation of means	Inference testing:t-test	exposed 4.75 (SD 2.6) vs non-exposed 4.3 (SD 1.1)	(p-value=0.394)	_	Serious

Thumerelle, 2003	37 (29+8) - Subpopulation	Viral pathogen only positive	Atypical bacteriae positive	Duration of hospitalization (in days)	presentation of means	Inference testing: Fisher exact and Wilcoxon test	viral pathogen exposed 4.3 (SD 2.3) vs M. pneumoniae exposed 6.25 (SD 3.2)	(p-value=0.096)	_	Serious
Response to Treatment										
Ducharme, 2016	965 - Entire population	Pathogen positive	Pathogen negative	Duration of symptoms (in ED - Proportion of children with severity score PRAM≥4, at disposition or 4 hours after oral corticosteroids)	presentation of proportions; OR	Multivariate logistic regression	exposed 119 (20.7%) vs non-exposed 55 (15.5%)	_	Adjustment for baseline PRAM, ipratropium bromide received, Caucasian ethnicity, and viral detection. OR (95%CI): 1.53 (1.05, 2.22)	Moderate
Ducharme, 2016	965 - Entire population	Pathogen positive	Pathogen negative	Duration of symptoms (in ED): Time to PRAM<=3 = consideration for discharge	HR	Cox proportional hazards model	_	_	Adjustment for baseline PRAM, perfect adherence to oral corticosteroids, viral detection, and sites. HR: 0.86 (95%CI 0.74, 0.99)	Moderate
Ducharme, 2016	613 - Subpopulation	Pathogen positive	Pathogen negative	Duration of symptoms (in days)	presentation of means; regression coefficients	Linear regression model	exposed 7.14 (SD 2.90) vs non-exposed 6.71 (SD 2.83)	_	Adjustment for asthma symptoms between episodes, Caucasian ethnicity, number of short courses or oral corticosteroids in the past year and viral detection. Coeficient (95%CI): 0.48 (0.01, 0.95)	Serious
Ducharme, 2016	965 - Entire population	Pathogen positive	Pathogen negative	Length of active treatment inhalation albuterol (in ED) (in hours)	presentation of means; Regression coefficients	Linear regression model	exposed 4.00 (SD 4.16) vs non-exposed 3.32 (SD 2.48)	_	Adjustment for baseline PRAM, delay between triage and oral corticosteroids, fever, number of albuterol received in the first hour, viral detection, and sites. Coeficient 0.10 (95% CI 0.03, 0.17)	Moderate
Ducharme, 2016	965 - Entire population	Pathogen positive	Pathogen negative	Duration of use of rescue beta2-agonist (in days)	presentation of means	Linear regression model	exposed 6.65 (SD 3.16) vs non-exposed 6.53 (SD 3.35)	_	_	Serious
Ducharme, 2016	965 - Entire population	Pathogen positive	Pathogen negative	Failure of emergency department treatment management (composite outcome)	presentation of proportions; OR	Logistic regression model	exposed: 110/579 (19%) vs non-exposed 46/354 (13%)	_	Adjusted for age, sex, baseline PRAM, viral detection (yes or no), salivary cotinine (values of <1 ng/mL, 1 ng/mL to <4 ng/mL, and ≥4 ng/mL),23 and oral corticosteroids dose (mg/kg): OR 1.61, 95%CI 1.06-2.45); adjusted for symptoms between exacerbations, fever, baseline PRAM, oxygen saturation, viral trigger, and sites: OR 1.57 , 95%CI (1.04-2.37) with p-value=0.0312.	Moderate
Ducharme, 2016	965 - Entire population	HRV positive	HRV negative	Failure of emergency department treatment management (composite outcome)	Presentation of proportions; OR	Logistic regression model	_	HRV exposed vs HRV non-exposed: OR (95%CI) : 0.96 (0.67, 1.39), (p=0.84) HRV/enterovirus exposed vs HRV/enterovirus non-exposed: OR (95%CI) : 1.00 (0.69, 1.44), (p=0.99)	_	Serious
Duenas 2016	169 - Entire population	M pneumoniae and virus positive	Virus alone positive vs M. pneumoniae and virus negative	Steroid requerement	presentation of proportions	Inference testing: X-qsuare, Fisher's exact	exposed 18 (85.7%) vs virus alone: 104/123 (84.6%) vs non-exposed 17 (68.0%)	(p-value=0.129)	_	Serious
Hanhan, 2008	35 - Entire population	M. pneumoniae positive	M. pneumoniae negative	Duration of continuous albuterol nebulization (in hours)	presentation of means	Inference testing: t-test or X-square	exposed 27.7 hours vs non-exposed 29 hours	(p-value =NS)	_	Serious
Kantor, 2016	155 - Subpopulation	HRV positive	Pathogen negative	Acute Severity Score (composite outcome)	regression coefficients	univariate and multivariate linear regression	_	Univariate coefficient: 3.65, (95% CI 2.32-4.98), (p-value<0.001)	Adjusted for age, sex, race, annual income, lung function, baseline asthma severity, symptom duration, medication adherence, total number of Immuno CAP postives, season. Multivariate coefficient: 3.34, (95% CI 2.24-4.43), (p-value<0.001). Interaction HRV infection and atopybaseline mouse IgE and Baseline Dust Mite IgE.	Serious
Kantor, 2016	155 - Subpopulation	HRV positive	Pathogen negative	Number of hours over which beta-agonist therapy was weaned (nitiation of therapy until intermittent treatments every 2 hours)	regression coefficient	univariate linear regression	_	13.90, (95%CI 8.14-19.66), (p-value<0.001)	_	Serious
Kato, 2011	33 - Subpopulation	HRV positive	RSV positive	Duration of systemic corticosteroid use (in days)	presentation of means	_	HRV exposed 4.7 (SD 2.4)vs RSV exposed 4.3 (SD 2.9)	_	_	Serious
Malka, 2015	66 - Entire population	Pathogen positive	Pathogen negative	change of FEV1 following albuterol (in %predicted)	presentation of means	Inference testing: X-square	exposed 24 (SD 3) vs non-exposed 36 (SD 3)	(p-value=0.002)	_	critical

Malka, 2015	66 - Entire population	Pathogen positive	Pathogen negative	FEV1 at baseline, at exacerbation and after initiation of prednisone	presentation of means	Linear regression model	Exposed 97.23 (SD 3.93) , 71.53 (SD 3.90), 93.07 (SD 3.99) vs non-exposed 97.72 (SD 4.41) , 75.54 (SD 4.38) , 95.39 (SD 4.58)	_	Adjustment for season. No differences were found between PCR (+) and PCR (-) groups in the measures of lung function studied across visits. (adjusted and with interaction term)outcome = adjusted means - no EM givendetected virus and visit	critical
Malka, 2015	66 - Entire population	Pathogen positive	Pathogen negative	duration of symptoms requiring rescue albuterol at moment of presentation with asthma exacerbation: median (first third quartile)	presentation of median (Quartiles)	Inference testing: Wilcoxon test	exposed 1(1,2)vs non-exposed 2(1,3)	(p-value=0.23)	_	critical
Ozcan, 2011	104 - Entire population	Pathogen positive	Pathogen negative	Systemic steroid requirement yes/no - (GINA guidelines)	presentation of proportions	Inference testing	Exposed 35 (62.5%) vs non-exposed 33 (68.8%)	(p-value=0.504)	_	Serious
Rueter, 2012	135 - Subpopulation	Pathogen positive	Pathogen negative	Number of doses of b2-agonist administration after 6, 12, and 24 hours	presentation of geometric means	linear regression model	Exposed 7 (6.4-7.5), 10 (9.4-11.1) and 15 (13.5-16.4) vs non-exposed: 7 (6.2-7.4), 11 (9.4-11.8) and 15 (13.6-17.5).	(For 6, 12 and 24 h: p-value = 0.768, p-value= 0.712, p-value= 0.626)	_	Serious
Rueter, 2012	135 - Subpopulation	HRV positive	HRV negative	Number of doses of b2-agonist administration after 6, 12, and 24 hours	presentation of geometric means	linear regression model	Exposed 7 (6.5-7.8), 10 (9.6-11.5) and 15 (13.7-17.0) vs non-exposed 7 (6.0-7.3), 10 (9.2-11.6) and 15 (13.4-17.3)	(For 6, 12 and 24 h: p-value=0.296, p-value=0.809, p-value=0.965)	_	Serious
Rueter, 2012	135 - Subpopulation	HRV positive	positive for any virus other than HRV	Number of doses of b2-agonist administration after 6, 12, and 24 hours	presentation of geometric means	linear regression model	Exposed 7 (6.6-7.8), 11 (9.6-11.6) and 15 (13.7-17.1) vs non-exposed 5 (4.7-6.1), 8 (6.9-9.9) and 12 (9.8-14.9)	For 6, 12 and 24 h: P = 0.015, P = 0.059, P =0 .106	At 6 hours: Adjusted for age and sex. p-value for HRV+ in multivariate model= 0.032. No interaction terms used.	Serious
Morbidity and Mortality										
Akturk, 2016	125 - Entire population	Pathogen positive	Pathogen negative	Chest X-ray finding: peribronchial infiltration, consolidation or atelectasis	presentation of proportions	Inference testing: X-square test, X-square with Yates' correction and Fisher's exact test	exposed 51 (48.1%) vs non-exposed 2 (10.5%)	(p-value=0.002)	_	Serious
Akturk, 2016	125 - Entire population	Pathogen positive	Pathogen negative	Duration of symptoms (in days)	presentation of median (range)	Inference testing : t-test, Mann–Whitney U	exposed 7 (4-20) vs non-exposed 4 (2-5)	(p-value<0.001)	_	Serious
Amin, 2013	130 - Entire population	Pathogen positive	Pathogen negative	Chest radiograph finding: consolidation	presentation of proportions	Inference testing: Mann-Whitney test	exposed 22/54 (40.7%) vs non-exposed 39.4% (30/76)	(p-value=1.000)	_	Critical
Amin, 2013	130 - Entire population	Pathogen positive	Pathogen negative	Chest radiograph finding: infiltration	presentation of proportions	Inference testing: Mann-Whitney test	exposed 32/54 (59.3%) vs non-exposed 46/76 (60.6%)	(p-value=1.000)	_	Critical
Amin, 2013	130 - Entire population	Pathogen positive	Pathogen negative	Death	presentation of proportions	Inference testing:Fisher's exact	exposed 6/54 (11.1%) vs 10/76 (13.2%)	(p-value=0.792)	_	Critical
Arden, 2010	39 - Subpopulation	HRV-A positve	HRV-C positive	Asthma cough score on day 21 and day 28 post admission (range 0-5)	presentation of median	Inference testing: Kruskal– Wallis test	HRV-A exposed d21: 2.0 (IQR 0– 3.0) and d28: 2.0 (IQR 0 – 2.0) vs HRV-C exposed d21: 0 (IQR 0-1.0) and d28: 0 (IQR 0-1.0)	d21: (p-value=0.04); d28: (p-value=0.04)	_	Serious
Arden, 2010	51 - Subpopulation	2 or more viruses positive	single virus positive	PACQLQ score: Paediatric Asthma Caregiver's Quality of Life Questionnaire score; on presentation	presentation of median	Inference testing: Kruskal– Wallis test	co-infection exposed 4.9 (IQR 3.9 – 5.5) vs single virus exposed 5.4 (IQR 4.5 – 6.0)	(p-value=0.04)	_	Serious
Arden, 2010	39 - Subpopulation	HRV-A positive	HRV-C positive	PACQLQ score: Paediatric Asthma Caregiver's Quality of Life Questionnaire score; on presentation and day 28	presentation of median	Inference testing: Kruskal– Wallis test	On admission: HRV-A exposed 5.2 (IQR 4.3 – 5.9) vs HRV-C exposed 5.5 (IQR 4.5–6.2); on day 28: HRV-A exposed 6.7 (IQR 6.3–7.0) vs HRV-C exposed 6.8 (IQR 6.4 – 7.0)	(admission p-value=0.50; d28 p-value=0.83)	_	Serious
Awasthi, 2012	44 - Entire population	C. pneumoniae positive	C. pneumoniae negative	Chest X-ray reading: Consolidation yes/no	OR	Inference testing	_	OR 18.29, (95 % CI 1.48-507.01), (p-value=0.01)	_	Serious
Bebear, 2014	168 - Entire population	M pneumoniae positive	M pneumoniae negative	Chest X-ray findings: consolidation yes/no	presentation of proportions	univariate (and multivariate) logistic	Exposed 1/10 (10%) vs non-exposed 19/145 (13%)	_	model not provided - reported as statistical non-significant differences	Serious
Ducharme, 2016	517 - Subpopulation	Pathogen positive	Pathogen negative	Parental functional status (Young Child's Asthma Flare-up on the Parents (ECAP)17 questionnaire - scale of 1 (best) to 7 (worst))	presentation of means; regression coeffcients	Regression model	exposed 4.60 (SD 1.17) vs non-exposed 4.32 (SD 1.22)	_	Adjusted for asthma symptoms between episodes, Caucasian ethnicity, dose of oral corticosteroids at discharge, viral detection, and sites. Coefficient (95% CI): 0.23 (0.01 0.44)	Serious
Duenas 2016	169 - Entire population	M pneumoniae	Virus alone positive vs M.	Community acquired pneumonia: clinical	presentation of proportions	Inference testing: X-square statistic	exposed 3 (14.3%) vs virus alone: 16/123 (13%) vs non-exposed 2 (8.0%)	(p-value=0.758)	_	Serious

		and virus positive	pneumoniae and virus negative	symptoms plus alveolar infiltrates on chest radiography		and Fisher's exact; Student's t-test for independent samples and the Mann– Whitney U-test				
Hanhan, 2008	35 - Entire population	M pneumoniae positive	M pneumoniae negative	Chest x-ray reading: presence of one or more infiltrates	presentation of proportions	Inference testing: t-test or X-square	exposed 13/15 (86%) vs non-exposed 7/20 (35%)	(p-value =0.002)	_	Serious
Jartti, 2007	50 - Subpopulation	HRV positive	HRV negative	Duration of cough (in days)	presentation of median	multivariate linear regression with backward stepwise	Duration of cough in days in rhinovirus pos, steroids treated: 2 (IQR0-7) vs in rhinovirus neg 6 (IQR 4-10). In rhinovirus pos non-treated: 10 (IQR 4-13) vs in rhinovirus neg non-treated 7 (IQR 3-12)	_	Univariate analysis interaction HRV and prednisolone: p-value=0.033; Multivariate analysis interaction HRV and prednisolone p-value=0.033. Adjustment for study drug, rhinovirus infection, study drug*rhinovirus infection. Duration of cough in rhinovirus pos group: RR 2.71, 95% CI 1.34–5.47, p = 0.0053. In non-rhinovirus group, RR 1.06, 95% CI 0.67–1.68, p = 0.80.	Serious
Jartti, 2007	50 - Subpopulation	HRV positive / Enterovirus positive	HRV negative / Enterovirus negative	Duration of dyspnea (in days)	presentation of median	multivariate linear regression with backward stepwise	-HRV pos, steroids treated: 0 (IQR 0-2) vs in HRV neg 4 (IQR 0-5). HRV pos non-treated: 2 (IQR 0-4) vs HRV neg non-treated 2 (IQR 0-3) - enterovirus pos, steroids treated: 2 (IQR 0-2) vs enterovirus neg 3 (IQR 0-4). Enterovirus pos non-treated: 3 (IQR 0-4) vs enterovirus neg non-treated 1 (IQR 0-3)	_	Interaction Rhinovirus infection and steroid treatment: 0.033; for enterovirus and steroid treatment: 0.89; Multivariate model HRV adjustment for study drug, rhinovirus infection, age, study drug*rhinovirus infection; In rhinovirus group, RR 4.31, 95% CI 1.12–16.64, p = 0.034. In non-rhinovirus group, RR 0.77, 95% CI 0.33–1.78, p = 0.54	Serious
Jartti, 2007	50 - Subpopulation	Enterovirus positive	Enterovirus negative	Duration of cough (in days)	presentation of median	multivariate linear regression with backward stepwise	enterovirus pos, steroids treated: 6 (IQR 5-11) vs enterovirus neg, steroid treated 4 (IQR 2-7); enterovirus pos non-treated: 7 (IQR 4-14) vs enterovirus neg non-treated 8 (IQR 4-12)	_	Univariate analysis interaction enterovirus and prednisolone: p=0.10; multivariate analysis interaction and prednisolone: p=0.49.	Serious
Kantor, 2016	155 - Subpopulation	HRV positive	Pathogen negative	Peak Expiratory Flow (PEF), percent predicted	regression coefficients	univariate linear regression	_	-16.19 (95%CI -22.11-(-10.27)), (p-value<0.001)	_	Serious
Kato, 2011	33 - Subpopulation	HRV positive	RSV postive	Wheeze period (days)	presentation of means	Inference testing	HRV exposed 6.5 (SD 4.1) vs RSV exposed 4.8 (SD 1.8)	_	_	Serious
Leung, 2010	209 - Entire population	HRV positive	HRV negative	Duration of fever (in days)	presentation of means	Inference testing: t-test	exposed 0.40 (SD 0.63) vs non-exposed 0.53 (SD 0.93)	(p-value=0.248)	_	Serious
Leung, 2010	209 - Entire population	HRV positive	HRV negative	FeNO (ppb); FEV1 (% predicted) ; FVC (% predicted); FEV1 to FVC ratio; PEF (L/min)	presentation of means	Inference testing: t-test	FeNO exposed 31.7 (20.3) vs non-exposed: 62.4 (44.8) FEV1 exposed 68.1 (28.1) vs non-exposed 74.3 (20.4) FVC exposed 70.7 (27.1) vs non-exposed 84.0 (33.4) FEV1 to FVC ratio exposed 0.82 (0.11) vs non-exposed 0.81 (0.27) PEF, L/min: exposed 156 (57) vs non-exposed 201 (81)	p value=0.018;p value=0.569; p value=0..251; p value=0..800; p value=0..104	_	Serious
Nagayama, 1999	212 - Entire population	Bacteria positive	Bacteria negative	Pneumonia: abnormal shadow in their chest roentgenogram on admission	presentation of proportions	Inference testing	exposed 12/43 vs non exposed 22/169	reported comparison: Pneumonia in bacteria exposed vs pneumonic cases in the presence of bacteria; P = 0.0175)	_	Serious
Ou, 2008	316 - Subpopulation	M pneumoniae positive	M pneumoniae negative	Chest X-ray reading as patchy or infiltration	presentation of proportions	Inference testing: t-test	In known asthma patients: 1. Patchy: 8/42 (19%) vs non-exposed 25/146 (17%) ; 2. Infiltration exposed 41/42 (97%) vs non-exposed 124/146 (85%) In first time asthma patients: 3.Patchy in Mycoplasma exposed: 8/57 (13%) vs non-Mycoplasma exposed: 12/71 (17%) ; 4.Infiltration in Mycoplasma exposed: 53/57 (93%) vs non-Mycoplasma exposed: 65/71 (92%)	1.(p-value=0.73) 2. (p-value=0.97) 3. (p-value=0.65) 4.(p-value=0.76)	_	Serious
Ozcan, 2011	104 - Entire population	Pathogen positive	Pathogen negative	Time to the alleviation of exacerbation (days)	presentation of means	Inference testing	exposed 2.02 (SD 1.3) vs non-exposed 2.12 (SD 1.3)	(p-value=0.709)	_	Serious
Thumerelle, 2003	82 - Entire population	Pathogen positive	Pathogen negative	Radiographic opacity	presentation of proportions	Inference testing: X-qsuare	exposed 24% vs non-exposed 29%	(p=0.51)	_	Serious
Thumerelle, 2003	82 - Subpopulation	Viral pathogen only	Atypical bacteriae positive	as measured by 1 radiologist in all patients	presentation of proportions	Inference testing: Fisher exact and Wilcoxon test	exposed 24% vs non-exposed: 50%	(p=0.157)	_	Serious

Thumerelle, 2003	82 - Entire population	Pathogen positive	Pathogen negative	Improvement at 48h: total, partial, none	presentation of proportions	Inference testing: X-qsuare	exposed 43%, 54%, 3% vs non-exposed 63%, 37%, 0%	(p-value=0.131)	_	Serious
Thumerelle, 2003	82 - Entire population	Pathogen positive	Pathogen negative	complete recovery at follow up visit	presentation of proportions	Inference testing: X-qsuare	exposed 78% vs non-exposed 88%	(p-value=0.085)	_	Serious
Thumerelle, 2003	82 - Subpopulation	Viral pathogen only	Atypical bacteriae positive	Improvement at 48h: total, partial, none	presentation of proportions	Inference testing: Fisher exact and Wilcoxon test	exposed 45%, 55%, 0% vs non-exposed 37.5%, 50%, 12.5%	(p-value=0.297)	_	Serious
Thumerelle, 2003	82 - Subpopulation	Viral pathogen only	Atypical bacterial infection	complete recovery at follow up visit	presentation of means/medianpresentation of proportions	Inference testing: Fisher exact and Wilcoxon test	Complete recovery at follow-up visit: in general pathogen exposed: 86% vs non-exposed: 50%; p=0.028	(p-value=0.028)	_	Serious

ADV: adenovirus, CoV: coronavirus, C. pneumoniae: Chlamydia pneumoniae, EV: enterovirus; H1N1: influenza virus Hemagglutinin Type 1 and Neuraminidase Type 1, HBoV: bocavirus, H. influenzae: Haemophilus influenzae, hMPV: human metapneumo virus, HRV: human rhinovirus, HRV-A: human rhinovirus A, INF: influenza virus, INF-A: influenza A virus, INF-B: influenza B virus, M. pneumoniae: Mycoplasma pneumoniae, PIV: parainfluenza virus

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Chapter 4: Integration of the reviewed literature into new evidence on the association between respiratory pathogens and asthma exacerbation outcomes research

As a result of our systematic search, we found only a limited number of studies that had investigated the association between pathogens and clinically important outcomes, comparing children with an asthma exacerbation with specific pathogens to those without. The quality of the association assessment was low in general. Other limitations were: the large number of different pathogens (exposures), an insufficient number of adequately powered studies in our review to provide conclusive evidence on pathogen-specific effects (except for HRV-C) and limited statistical analysis (inference testing only with presentation of p-values), not thoughtful on confounders or interaction. Prior narrative reviews have suggested, however, that the clinical presentation of asthma exacerbation is pathogen-dependent and does not depend only on the presence or absence of any pathogen (39, 52). Additionally, the heterogeneity in outcomes and outcome definitions prevented pooling of the data. Our systematic review concluded that there was a possible association between exacerbation severity and HRV-C species (study of Bizzintino et al.) (72). The clinical importance of a one-point increase on a severity scale (min 0 and max15) may however be limited. Evidence with a low-to-moderate risk of bias of the association between respiratory pathogens and health care utilization and other outcomes of morbidity was lacking in the reviewed literature. The DOORWAY study (Ducharme et al (13)) was included in our systematic review and found an association between the presence of a pathogen in general (any pathogen) and an increased risk of treatment failure, without studying the pathogen-specific association with treatment response. Moreover, the study analysis did not have the objective to

investigate the association between the presence of a pathogen and the severity of asthma exacerbation on presentation.

For the main analysis of my thesis and for my second manuscript, I used the primary data of the DOORWAY study, which tested for the presence of 23 different respiratory pathogens in a large prospective cohort of 1012 enrolled asthmatic patients 1-17 years old. Ducharme et al reported a discrepancy of about 15% between the proportion of asthmatics for whom there was a perception (by parents or healthcare workers) that the exacerbation was triggered by a respiratory infection (likely viral) and the proportion of laboratory-confirmed viral respiratory tract infection. Given this difference, one would wonder if other aetiologies, such as atypical bacteria would not be a trigger of disease. We carried out a laboratory diagnostic sub-study, testing the stored respiratory specimens of included patients for additional pathogens and species. Combining the data from the primary study (both primary and derived variables) and the sub-study, I first describe the pathogen prevalence in this large cohort, followed by an investigation of the pathogen-specific association between laboratory-confirmed pathogen and the clinical outcomes of exacerbation severity and treatment failure. In the next chapter, I elaborate on the study methods used in my secondary analysis of the DOORWAY data, followed by the presentation of my second manuscript and a short result section presenting some additional analysis results. The manuscript is formatted for submission to *Clinical Infectious Diseases*.

Chapter 5: Descriptive study of burden of Respiratory Pathogens in children with moderate or severe asthma exacerbation presenting to the ED, association with exacerbation severity on presentation and ED treatment failure

Our aims were 1) to describe the burden of viral and bacterial respiratory pathogens in a large cohort of children presenting to the ED with asthma exacerbation over a 3-year period, 2) to determine the association between the presence of a laboratory-confirmed respiratory pathogen and (i) exacerbation severity at presentation and (ii) ED treatment failure.

I. Methodology

1. Study Setting, Design and Data Source

For this second manuscript, I used the data of the DOORWAY study (registered at Clinicaltrials.gov – NCT02013076), a prospective multi-center cohort study of children, age 1-17 years old, presenting with an asthma exacerbation between January 2011 and December 2013, to 1 of 5 Canadian EDs in the provinces of Quebec (Centre Hospitalier Universitaire Sainte-Justine, McGill University Health Centre, Centre Hospitalier Universitaire de Laval) and Ontario (Children's Hospital of Eastern Ontario, London Health Sciences Centre). Inclusion and exclusion criteria are detailed in the manuscript that follows. The severity of exacerbations was determined using the validated Pediatric Respiratory Asthma Measure (PRAM) score, ranging from 0 (absence of symptoms) to 12 (most severe symptoms), scores of 4 to 12 are considered moderate to severe (73). The DOORWAY study focused on patients with at least moderate exacerbations (PRAM ≥4). Of the 1197 eligible children, 185 (15%) declined to participate in the primary study.

Their baseline characteristics did not differ from the children included in the study. A total of 1,012 children were enrolled in the initial study, of which 973 participants were included in the primary analysis after the removal of children who withdrew from the study (n=3), those with protocol deviations (n=31) and diagnostic uncertainty or ineligibility (n=5). All children received a per-protocol defined dose of corticosteroids (1 or 2 mg/kg of oral prednisone or prednisolone or 0.3mg/kg of oral dexamethasone) and severity-specific inhaled bronchodilator treatment with salbutamol, with or without ipratropium bromide. All enrolled participants underwent nasopharyngeal swabs for testing of respiratory pathogens by automated microarray detection. The test detected 23 common respiratory viruses, including the H1N1 pandemic strain and species of enterovirus in general, as well as rhinovirus A and B. The study found that, in children presenting to the ED with moderate to severe asthma exacerbation; viral triggers (19% vs 13%; OR 1.57, 95%CI 1.04-2.37), fever (24% vs 15%; OR 1.96, 95%CI 1.32-2.92), baseline PRAM score (OR 1.38 per 1-point increase, 95%CI 1.22-1.56), oxygen saturation below 92% (50% vs 12%; OR 3.94, 95%CI 1.97-7.89) and asthma symptom chronicity (21% vs 16%; OR 1.73, 95%CI 1.13-2.64) were associated with an increased risk of failure in ED management of asthma. In contrast, age, salivary cotinine concentration and oral corticosteroids dose were not, as in the study defined, statistical significantly associated with management failure. Failure of ED management was defined as either hospital admission for asthma, an ED stay for asthma of 8 hours or more after administration of oral corticosteroids, or a return visit within 72 hours of discharge leading to hospital admission or prolonged ED stay of 8 hours or longer, for asthma.

All DOORWAY study patients with a valid respiratory specimen were included in my sub-study (n=958). To assess the association with treatment failure, children with a protocol deviation were

excluded from our analysis (remainder n=932). Details can be found in the flow chart in the following manuscript. (Figure 1)

2. Exposure variables

The identification of a laboratory-confirmed pathogen (viral or atypical bacteria) in respiratory specimens (i.e., nasopharyngeal swabs or aspirates systematically procured in the first hour following patient study inclusion) was considered a positive exposure. Single specific pathogen exposures and aggregate exposure groups were used in different models. The laboratory diagnostic methods included in the initial study included the detection of 23 respiratory viruses using a validated multiplex RT-PCR microarray hybridization assay (74). We performed in addition a commercial multiplex PCR assay (Seeplex PneumoBacter ACE Detection, Seegene, ON, Canada (75)) that tested for 3 additional pathogens: the atypical bacteria *Mycoplasma pneumoniae and Chlamydophila pneumoniae; and Bordetella pertussis*. Although this test also identifies *Legionella pneumophila, Streptococcus pneumoniae* and *Haemophilus influenzae,* we *a priori* decided to disregard the latter results because *Legionella* is not described in the literature as a common trigger of asthma exacerbations and given the poor specificity of the diagnostic assay for *Streptococcus sp.* and *Haemophilus sp.*, which would have resulted in substantial misclassification of patients colonized with other non-pathogenic species (76).

Specimens that were positive for enterovirus/rhinovirus in the original study using the primary RT-PCR assay were further analyzed using a more specific RT-PCR assay (77) and a sequencing protocol (78) targeting the 5' noncoding region (NCR) of HRV A, B, and C and of enterovirus, including EV-D68 to type these pathogens up to species level.

Pathogens that had less than 10 positive cases were not independently investigated further.

Exposure to the different HRV species (HRV-A, HRV-B, HRV-C) was separately investigated

given our literature review that suggested a differential effect of the different species. RSV-A is more commonly circulating and has been suggested to result in more severe disease compared to RSV-B in the pediatric population (79, 80), but data are conflicting (81) and there are no data in children with asthma. For the coronaviridae, there are no data in the asthma literature suggesting a different clincial picture with infection by different species. In general, differences have only been described for Middle East respiratory syndrome-related coronavirus (MERS-CoV) and Severe acute respiratory syndrome-related coronavirus (SARS) species. Of the PIV species, PIV-3 is known to infect the lower respiratory tract. This resulted in the ascertainement of the following exposures in the different models: HRV-A, HRV-B, HRV-C, EV-D68, INF, RSV, hMPV, ADV, Cov and PIV.

All respiratory specimens that tested positive for any virus or atypical bacteria were coded as "respiratory pathogen" positive. A categorical exposure variable for HRV was also created as: HRV-positive, non-HRV pathogen positive, or pathogen negative. Co-infection was defined as the presence of ≥2 pathogens and a categorical variable: ≥2 pathogens, single pathogen positive or pathogen negative was created.

3. Co-variates

Additional covariates describing the population, potential confounders (15), mediators, and interaction terms included in the models were selected based on literature review. Co-variates were: age in years (continuous), sex, child atopy (based on parental report of a diagnosis or symptoms of allergic rhinitis and eczema, as documented by the International Study of Asthma and Allergies in Childhood questionnaire (21)), asthma phenotype (intermittent or persistent (15)), oral corticosteroid use in the preceding 12 months as a marker of morbidity, asthma control (measured with the 6-point Asthma Quiz for Kidz (82)), asthma controller use (daily, only when

sick, none), season (based on calendar), upper respiratory tract infection (URTI) symptoms at index visit (clinical diagnosis), fever (at ED presentation), pneumonia (physician diagnosis based on clinical signs and chest X-ray), tobacco exposure (categorical variable based on saliva cotinine levels sampled after study inclusion (83)), average family income quantile (based on postal-code census data, as a proxy for socioeconomic characteristics (84)), and study site. A more detailed description can be found in the primary study (13).

Atopy referred to any of the 4 symptoms in the past medical history, namely: rhinitis or hay fever; sneezing, runny or blocked nose in the absence of cold; itching, rash or eczema, as documented by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (21). The assumption was made that when parents or patients answered "I do not know" on 1 out of the 4 questions and "yes" to none of the 4 questions, that no atopy was present.

Previous morbidity and asthma severity was assessed by systemic corticosteroid use in the previous 12 months. This refers to a previous exacerbation of moderate to severe severity, and is one of the strongest predictors of future exacerbation (85, 86).

The variable season represents the circulation of pathogens and partly represents differences in environmental factors like humidity and temperature.

There were 5 sites included in the study and it was used as a categorical variable. However, one site (Centre Hospitalier Universitaire Laval (CHUL) in Quebec) only enrolled 5 patients and this site was merged with the Centre Hospitalier Universitaire Sainte-Justine (CHUSJ) site. This was chosen based on geographical proximity and location within the same province (Quebec) under the same provincial health care system as Sainte-Justine. The Montreal Children's Hospital, the third centre within the province of Quebec, was allowed by the independent data monitoring committee to use a corticosteroid dose of 1mg/kg (as per their site standard treatment protocol), whereas all

other sites administered 2 mg/kg (max 50 mg) of oral prednisone/prednisolone and thus Laval and Sainte-Justine used equal corticosteroid dosing.

Other variables of interest that could not be included were: BMI z-score, because the proportion of missing values for BMI was 42.4% and specific IgE with >50% missing values.

Fever, physician-diagnosed URTI and pneumonia were considered as potential mediators of inflammatory response in the presence of a respiratory pathogen. Missing data ranged from 0% to 10% and are presented in detail in Table 2 of the manuscript.

4. Outcomes

The prevalence of respiratory pathogen positive specimens diagnosed by PCR was used as the outcome in the descriptive analysis. We used the PRAM score documented at study inclusion as the outcome for the second objective (severity), using the PRAM-score as a left truncated continuous variable and as a binary variable, that is, moderate (PRAM score 4-8) vs. severe (PRAM score 8-12) exacerbation. For the third objective, the composite outcome was ED treatment failure defined as hospital admission for asthma, treatment in the ED lasting 8 hours or more after corticosteroid administration, or a return visit to the ED within 72 hours of discharge leading to hospital admission or with prolonged ED stay of 8 hours or longer. This outcome was the same outcome as was used as the primary outcome in the DOORWAY study and was coded as a variable in the primary DOORWAY analysis.

5. Statistical Analysis

Descriptive statistics, using standard summary statistics (frequency counts and rates), were computed for the presence of respiratory pathogens, describing the prevalence of each separate respiratory pathogen tested in the study population. The denominator used was the total number of specimens tested.

Both linear and logistic regression models were used to investigate the association between respiratory pathogens and exacerbation severity, using the continuous outcome PRAM score in the former and the binary outcome, that is, moderate vs. severe exacerbation in the latter. Logistic regression was used to investigate the association between respiratory pathogens and the binary outcome treatment failure. Study site was used first as an independent covariate and then as a cluster, using a fixed effect 2-level hierarchical model. This resulted in the following exposure and outcome models:

a. Exposures:

- 1 model with any respiratory pathogen vs. no respiratory pathogen (reference)
- 1 model with rhinovirus positive vs. non-rhinovirus positive vs. pathogen negative (reference)
- 1 model per categorical specific pathogen exposure HRV-A, HRV-B, HRV-C, RSV, INF, hMPV, PIV and EV-D68; compared to "other pathogen positive" vs. pathogen negative (reference) (8 models in total)
- 1 model with co-infection vs. single pathogen (reference) vs. pathogen negative

B. Outcomes:

- 1 model using PRAM-score as a binary outcome moderate (PRAM 4-8) vs. severe (PRAM 9-12) exacerbation
- 1 model using PRAM-score as continuous outcome (as sensitivity analysis)
- 1 model using treatment failure as a binary failure yes vs. no failure

C. Cluster handling (cluster = study site)

- 1 model using site (the cluster) as an independent covariate

- 1 model using mixed multilevel model using sites as a cluster- hierarchical 1 level/fixed effect (as sensitivity analysis)

No correction for multiple testing was applied, not trading type 1 for type 2 errors (87).

Confounding assessment was performed without statistical testing(88). A directed acyclic graph (DAG) was drawn to determine confounders (89), mediators (consequences of the exposure) and colliders. (Figure 1 and 2)

Figure 1. - DAG exacerbation severity

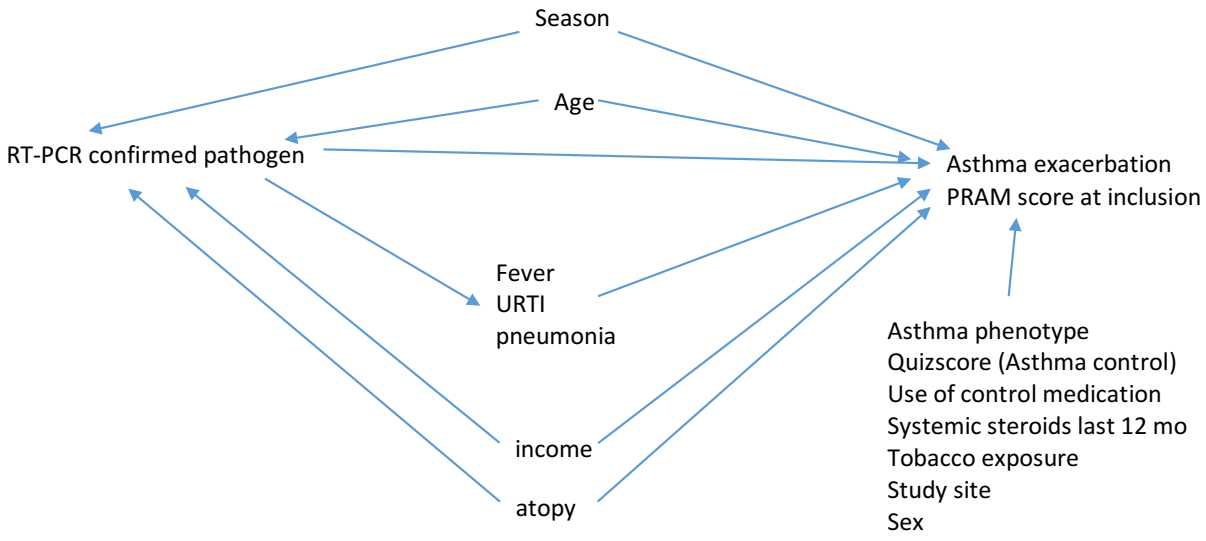

Fig . Directed acyclic graph showing the putative relationships between exposure RT-PCR confirmed pathogen and outcome asthma exacerbation PRAM score at inclusion.

Figure 2. - DAG treatment failure

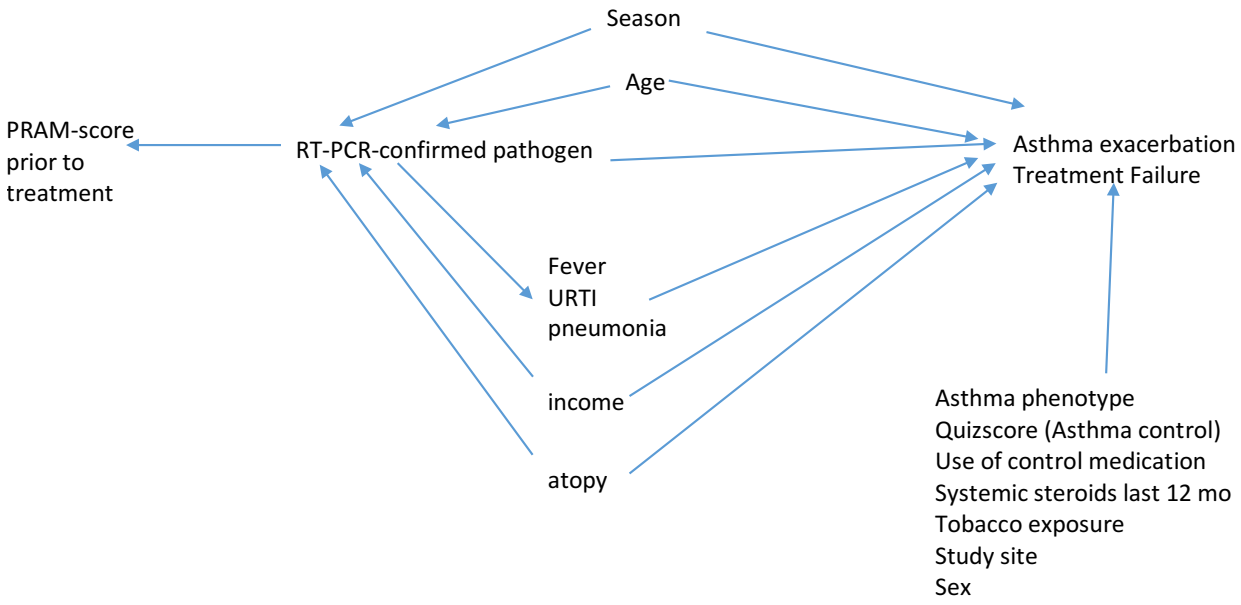

Fig . Directed acyclic graph showing the putative relationships between exposure RT-PCR confirmed pathogen and outcome asthma exacerbation treatment failure.

Patients with missing data in the included variables were excluded from the analysis. Most variables were complete. Missing data ranged up to 10%, with the highest proportion of missing found for the variable "income". Multiple imputations would have been an alternative approach to handle these 10% missing data. Collinearity was investigated using correlation matrix command in STATA(90). Interaction between exposures and atopy, allergic rhinitis, income and the possible mediators fever, pneumonia and URTI was assessed on the additive scale (91).

Model fit was assessed by the area under the curve (AUC), presenting receiver operating characteristic (ROC) curves (92) for the models using a binary outcomes. The larger the AUC, the better the model discriminates patients with the outcome from those without the outcome (88). AUC values are graded as: 0.70-0.80: fair discrimination and 0.8-0.90: good discrimination. For

the linear models using the continuous variable PRAM-score, model fit was assessed using R-squared (R^2), which is the proportion of variance in the dependent variable that can be explained by the independent variables.

Results of regression models are presented as predicted probabilities of the outcome (with their 95%CI) comparing specific pathogen exposed vs. non-exposed children, representing the average marginal effect (AME) across the total study population (93-95). This provides us with the predictive value of the outcome across all values of variables included in the model, reflecting the weighted average over the distribution of confounders and is an equivalent to estimates obtained by standardizing to the total population(94). A risk difference (RD) that did not include the null (0%) in its 95% CI was considered statistically significant and interpreted towards its clinical importance. Absolute risks and risk differences are presented with their confidence interval. OR would give a biased estimate given the outcomes severe exacerbation and treatment failure were not rare in this study population (96). We also prefer absolute risks and absolute comparisons above relative comparisons, given their more realistic representation of the magnitude of effects. Only the risk estimates of the investigated exposure effect measures and not for all the in the models included covariates are presented (97).

All analysis was performed using STATA 13 (StataCorp. College Station, TX) and R version 3.2.1 (www.r-project.org).

II. Manuscript

Respiratory pathogens in children with acute moderate and severe asthma: association with exacerbation severity and response to therapy.

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Keywords: asthma, children, respiratory pathogens, exacerbation severity, treatment

Running title: Asthma exacerbation and respiratory agents

Summary: Children presenting to the emergency department with moderate/severe exacerbation with a laboratory-confirmed respiratory pathogen did not display greater severity, but suffered an increased risk of treatment failure. Respiratory syncytial virus, influenza, and parainfluenza were particularly associated with treatment failure.

Abstract

Background: Asthma exacerbations are triggered by respiratory pathogens, but their impact on exacerbation severity and treatment response remains unclear. We investigated the association between respiratory pathogens and both baseline exacerbation severity and emergency department (ED) treatment failure.

Methods: We performed a secondary analysis of the DOORWAY prospective cohort study of children (1-17 years) presenting to the ED with moderate/severe asthma exacerbations, treated with bronchodilators and oral corticosteroids. Nasopharyngeal specimens were analyzed by RT-PCR for 27 respiratory pathogens. Linear and logistic multivariate regressions were used to estimate average marginal effects (absolute risks and risk differences (RD)).

Results: Of 958 patients, 591 (61.7%) were positive for ≥1 pathogen; rhinovirus (HRV) was the most prevalent (29.4%). Pathogens were not associated with higher severity on presentation. The overall risk of treatment failure was 16.9% (156/924). The presence of any pathogen or of a non-HRV pathogen increased the risk by 8.2 percentage points (95%CI 3.3%; 13.1%) and 13.1 percentage points (95%CI 6.4%; 19.8%), respectively (risk in the absence of a pathogen: 12.5%). Respiratory syncytial virus (RSV), influenza, and parainfluenza virus (PIV) were positively associated with treatment failure with RDs (95%CI) of 8.8% (0.4%; 17.2%), 24.9% (4.7%; 45.1%), and 34.1%; (7.5%; 60.7%), respectively.

Conclusions: Although respiratory pathogens were not associated with higher severity on presentation, they were associated with a higher risk of treatment failure, particularly in the presence of RSV, influenza and PIV. This supports influenza prevention in asthmatic children, pathogen identification on presentation, and exploration of treatment intensification for infected patients at higher risk of treatment failure.

Introduction:

Exacerbations constitute the largest burden of disease in children with asthma [1], with 60-80% being triggered by respiratory pathogens [2]. The use of reverse transcriptase polymerase chain reaction (RT-PCR) has facilitated the detection and our understanding of the epidemiology of respiratory pathogens in this population [3], with a growing interest in rhinovirus (HRV), the most frequently identified virus during exacerbations. Some reports have suggested the association between HRV-C and severe asthma exacerbations and hospitalizations [4]. Recently, enterovirus-D68 (EV-D68), has also been reported in outbreaks of severe respiratory complications in children with asthma [5]. Influenza (INF)'s role in asthma exacerbation severity and health care utilization, on the other hand, remains controversial [6, 7]. In addition, respiratory syncytial virus (RSV), parainfluenza virus (PIV) and other pathogens, including atypical bacteria such as *Mycoplasma pneumoniae*, have been associated with exacerbations[8], but only a limited number of studies have investigated the impact of specific pathogens on the severity of exacerbations [9] and none have primarily addressed treatment response.

Evidence-based emergency department (ED) management comprising the combination of inhaled bronchodilators and systemic corticosteroids[10-12] for moderate or severe asthma exacerbations have been shown to reduce the risk of hospitalization[13]. Ducharme et al. [14] investigated determinants of treatment failure in children with moderate or severe exacerbations presenting to the ED in the DOORWAY study, the largest cohort of its kind. The detection of a respiratory virus was associated with ED treatment failure. However, pathogen-specific effects were not investigated. Quantifying the impact of any and of specific respiratory pathogens would guide infection prevention interventions in children with asthma and focus efforts on respiratory pathogen diagnosis at presentation to the ED, to identify children at higher risk of treatment failure

in whom treatment intensification may be considered.

In children presenting to the ED with a moderate or severe asthma exacerbation, our aims were to ascertain the association between the presence of a laboratory-confirmed respiratory pathogen and both the severity of exacerbation on presentation and the risk of ED treatment failure.

Methods:

Data Sources, Study Design and Participants:

This study is a secondary analysis of the DOORWAY study (Clinicaltrials.gov: NCT02013076), a multicenter prospective cohort study of children (1-17 years) with moderate or severe asthma exacerbation, presenting to one of five participating Canadian pediatric EDs, [14]. Briefly, the original study's objective was to identify determinants associated with ED management failure after standardized therapy. Children were eligible if they were 1 to 17 years of age, had a physician diagnosis of asthma based on a previous episode with airflow obstruction with response to asthma medication, ≥3 wheezing episodes (if <2 years) or previous diagnostic lung function test results who presented to the ED and had a physician diagnosis of moderate or severe exacerbation. An independent committee adjudicated cases with diagnosis uncertainty. Severity of exacerbation was determined using the validated 12-point Pediatric Respiratory Asthma Measure (PRAM) score, with moderate and severe scores ranging from 4-7 and 8-12, respectively [15]. Patients were excluded if they presented with a suspicion of bronchiolitis or foreign body aspiration, another chronic respiratory disorder, hypersensitivity to salbutamol, ipratropium bromide or prednisolone or had a contra-indication to oral steroids. All children received a standardized dose of oral corticosteroids (2 mg/kg of oral prednisone or prednisolone or 0.3mg/kg of oral dexamethasone) and severity-specific inhaled bronchodilator treatment with salbutamol; ipratropium bromide was added in those with severe exacerbations. All eligible DOORWAY patients with a valid

respiratory specimen were included in this ancillary study.

Respiratory specimen pathogen testing – Exposure measures:

A nasopharyngeal aspirate or swab (Flocked swab, Copan Diagnostics, CA, USA) was systematically procured within one hour of study inclusion, placed in 3 mL of viral transport media (UTM, Copan Diagnostics) and frozen at -80^{0}C. Adenovirus (ADV B, C, and D), Coronavirus (CoV 229E, HKU1, NL63, OC43), EV (A, B, C, D, including EV-D68), INF (A, including pH1N1 and B), PIV (1, 2, 3, and 4), hMPV (A and B), HRSV (A and B) and HRV (A and B) were investigated using a validated multiplex RT-PCR microarray hybridization assay [16]. Nucleic acids from respiratory specimens were also tested using a commercial multiplex PCR assay (Seeplex PneumoBacter ACE Detection, Seegene, ON, Canada [17]) to identify the atypical bacteria *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae, and Bordetella pertussis.* Although this test also identifies *Legionella pneumophila, Streptococcus pneumoniae* and *Haemophilus influenzae,* we *a priori* decided to disregard the latter results because *Legionella* is not described in the literature as a common trigger of asthma exacerbations and given the poor specificity of the diagnostic assay for *Streptococcus sp.* and *Haemophilus sp.*, which would have resulted in substantial misclassification of patients colonized with other non-pathogenic species [18].

All specimens that tested positive for enterovirus/rhinovirus probe on the microarray assay [16], but negative for type-specific enterovirus or rhinovirus (n=302) and those that tested negative for the enterovirus/rhinovirus probe but positive for one of the enterovirus/rhinovirus type-specific probes (n=20), were further tested using RT-PCR and sequencing [19] targeting the 5' noncoding region (NCR) of HRV A, B, and C and of enteroviruses including EV-D68. For samples with undetermined results by RT-PCR and sequencing (n=16), results from the microarray analysis

were used. Respiratory specimen results were interpreted blinded to the clinical outcome. Exposure variables were as follows: respiratory specimens that tested positive for either virus or atypical bacteria were coded as "pathogen positive". Co-infection was defined as the presence of ≥2 pathogens compared to a single pathogen and no pathogen.

Outcome measures :

The PRAM score on presentation determined the exacerbation severity and was analyzed alternatively as a left truncated continuous variable and as a binary variable where a PRAM of 4-7 indicated moderate and 8-12, severe exacerbation. ED management failure was defined as hospital admission for asthma, treatment in the ED lasting 8 hours or more after corticosteroid administration, or a return to the ED within 72 hours of discharge leading to hospital admission or prolonged ED stay (8 hours or longer).

Data analysis:

The prevalence of each separate respiratory pathogen was described using frequency counts and rates. Pathogens with less than 10 positive cases were aggregated. Covariates identified in the literature were explored as potential confounders [20], mediators, and interaction terms, based on a directed acyclic graph [21]. A detailed description of covariates can be found in the primary study [14]. Briefly, covariates included in the models were: age in years (continuous), sex, child atopy (based on parental report of a diagnosis or symptoms of allergic rhinitis and eczema, as documented by the International Study of Asthma and Allergies in Childhood questionnaire [22]), asthma phenotype (intermittent or persistent [20]), oral corticosteroid use in the preceding 12 months as a marker of morbidity, asthma control (measured with the 6-point Asthma Quiz for Kidz [23]), asthma controller use (daily, only when sick, none), season (based on calendar), upper respiratory tract infection (URTI) symptoms at index visit (clinical diagnosis), fever (at ED presentation), pneumonia (physician diagnosis based on clinical signs and chest X-ray), tobacco

exposure (categorical variable based on saliva cotinine levels sampled after study inclusion [24]), average family income quantile (based on postal-code census data, as a proxy for socioeconomic characteristics [25]), and study site. Potential interactions between exposures and atopy, allergic rhinitis, income and the possible mediators fever, pneumonia and URTI were assessed on the additive scale. Linear and logistic regressions were used to investigate the association between pathogens and exacerbation severity using the baseline PRAM as continuous or dichotomous outcome, respectively. Logistic regression was used to investigate the association with treatment failure. This resulted in one model where the exposure was any pathogen vs. no pathogen, one model for HRV positive vs. non-HRV positive vs. pathogen negative, 8 models for each specific pathogen exposure (HRV-A, HRV-B, HRV-C, RSV, INF, hMPV, PIV and EV-D68 as categorical variable vs. "any other pathogen positive" vs. pathogen negative) using pathogen negative as the reference , and one model comparing co-infection with single pathogen. Study site was used first as an independent covariate and then as a cluster, using a fixed effect 2-level hierarchical model. Results of regression models are presented as predicted probabilities of the outcome (absolute risks (AR) and risk differences (RD) with their 95% confidence intervals (CI)) and representing the average marginal effect across the total study population [26, 27]. Missing data for covariates ranged from 0% to 10%. Patients with missing data for variables included in the final models were excluded from the analysis. Model fit was assessed by the area under the curve (AUC) [28] for logistic, and with R-squared for linear, regression. No correction for multiple testing was applied [29]. Analyses were performed using STATA 13 (StataCorp. College Station, TX) and R version 3.2.1 (www.r-project.org).

Results:

Of 1012 patients enrolled in the DOORWAY study, 958 were included in the assessment of the association between respiratory pathogens and the severity of exacerbation on presentation and 924 in the association with ED treatment failure. (Figure 1)

Distribution of respiratory pathogens; patient and exacerbation characteristics:

Of the 958 respiratory specimens tested, 591 (61.7%) were positive for ≥1 pathogen, with co-infection present in 81 specimens (8.5%) – RSV and CoV were the most frequent co-pathogens (n=13). The most prevalent pathogen was HRV (n=282, 29.4%) and HRV-C the most frequent species (n=174, 18.2%), followed by RSV (n=171, 17.9%). Only 2 patients were diagnosed with *Mycoplasma pneumoniae* (Table 1).

Compared to those without, children with a laboratory-confirmed pathogen were younger (median [interquartile range] age: 2 [1-5] vs. 4 [2-7] years), had higher tobacco exposure (cotinine levels ≥ 4ng/ml in 6.8% vs. 3.8%) and were only slightly more likely to present with fever: 29.4% vs. 24.2%, respectively (Table 2). Compared to children who were not HRV-positive, children who were HRV positive were less often febrile and less frequently diagnosed with pneumonia. Although most exacerbations with a laboratory-confirmed pathogen occurred in the fall (34%) or winter (34.0%), the seasonal distribution of specific respiratory viruses differed markedly (Figure 2).

Association between pathogen and exacerbation severity on presentation:

The proportion of children presenting with a severe exacerbation was 33.3% (95%CI 30.3%; 36.3%). Using PRAM as a binary outcome, no positive association was found between the presence of any respiratory pathogen and severe vs. moderate exacerbation. The adjusted absolute risk (95%CI) of a severe exacerbation was 32.4% (29.0%; 35.8%) in the presence of and 38.3% (33.6%; 43.0%) in the absence of a pathogen, representing a RD of -5.8% (-11.8; 0.01) (Figure 3A

& C; Appendix 1). Although the risk of severe exacerbation in HRV-positive children was similar to that of no pathogen, the presence of a non-HRV pathogen was associated with a 12.9 percentage point (95%CI -19.5; -6.3%) decrease in the adjusted risk of severe exacerbation. This appeared to be mainly due to the presence of hMPV and PIV that were associated with a lower risk of severe asthma with adjusted RDs (95%CI) of -13.6% (-23.0%; -4.3%) and -31.7% (-44.5%; -18.9%), respectively. No statistically significant association was found between severity on presentation and RSV, INF, EV-D68, ADV, and CoV. (Figure 3 B & D; Appendix 1) Pathogen-specific risk did not change when stratifying for co-infection or when using a hierarchical model with site as a cluster. The association between exposures and severity was also investigated using PRAM-score as a continuous outcome, repeating all the exposure models, which confirmed these results.

Association between pathogen and treatment failure

Overall, 156 of 924 included participants (16.9%, 95%CI 14.5%; 19.3%) experienced treatment failure. The presence of any respiratory pathogen compared to no pathogen was associated with a 8.2 percentage points (95%CI 3.3%; 13.1%) increase in the risk of treatment failure from AR (95%CI) 12.5% (9.0%; 16.0%) to 20.7% (17.4%; 24.1%) (Figure 4 A & C; Appendix 1). Although the presence of HRV and its species was not associated with treatment failure, there was a higher risk of treatment failure in children with a non-HRV infection, as a group, with an AR of 25.4% (95%C 19.8%; 31.0%) compared to no pathogen, resulting in an adjusted RD of 13.1% (95%CI 6.4%; 19.8%). Compared to no pathogen, specific pathogens RSV, INF and PIV were associated with higher risk (95%CI) of treatment failure namely, 21.4% (14.1%; 28.7%), 37.5% (17.8%; 57.2%), and 46.7% (20.4%; 73.0%), with RDs (95%CI) of 8.8% (0.4%; 17.2%), 24.9% (4.7%; 45.1%), and 34.1% (7.5%; 60.7%), respectively. hMPV had a RD of 8.0% (95%CI -1.6%; 17.6%). CoV, ADV, EV-D68 and the presence of a co-infection were not associated with an increased risk of treatment failure (Figure 4 B & D; Appendix 1). Pathogen-specific risk did not change when

stratifying for the presence of co-infection or when using a hierarchical model with site as a cluster.

Discussion

In this large prospective multi-center cohort study, we ascertained the presence of 27 different respiratory pathogens in children presenting to the ED with moderate or severe asthma exacerbations and their associations with exacerbation severity and treatment response. Of the > 900 respiratory specimens, 61.7% were positive for a viral pathogen and, in two cases, for atypical bacteria. No positive association was found between the presence of any pathogen and exacerbation severity on presentation. Despite standardized therapy with oral corticosteroids and severity-specific inhaled bronchodilators, the presence of any respiratory pathogen, and more specifically of a non-HRV pathogen, was associated with more treatment failure compared to non-infected counterparts.

Our study confirmed the high prevalence of HRV, in particular of HRV-C. HRV-C's high prevalence in children presenting with asthma exacerbation and its presumed association with exacerbation, as well as asthma-related hospitalization's peak in the fall have brought this virus at the forefront as a potential cause for more severe disease [9]. We found, however, that HRV-positive children had a similar risk of severe vs. moderate exacerbation, compared to children without a diagnosed pathogen. Unlike others, we were unable to confirm increased severity or treatment failure in HRV or HRV-C-infected children [30, 31]. HRV may play a role in the initiation of exacerbations severe enough to warrant health care contact and treatment with corticosteroids, but once infected, response to treatment is favourable. These results are in line with a subgroup analysis of a randomized controlled trials of children treated with corticosteroids,

in which those with HRV responded better to steroid treatment than those negative for entero/rhinovirus [32].

The presence of non-HRV pathogens, as a group, was associated with a larger proportion of children with a moderate, rather than severe exacerbation on presentation. The negative association appeared dominated by children who tested positive for hMPV (about 10%) and for PIV (only in 1.5% of patients,) who had a predominance of exacerbation of moderate severity. Yet, in contrast, children with non-HRV pathogens in general, particularly those with RSV (almost 18% of the study population), INF (2.5%), and PIV were associated with higher treatment failure in the ED, after adjustment for patient and exacerbation characteristics. Consequently, severity on presentation and response to treatment are two distinct dimensions, with the latter appearing as the most clinically important for patients and health care utilization.

By far, the most prevalent non-HRV pathogen was RSV. RSV was not associated with severe exacerbation on presentation, but was associated with a higher risk of treatment failure (RD 8.8%; (95%CI 0.4%; 17.2%)). This is supported by previously documented lowered response to steroids in asthmatic children infected with RSV compared to non-infected children[31]. This study was however limited in size. There is substantive literature on the lack of response to steroids in children with RSV bronchiolitis [33]. Our study excluded children <1 year of age and those with a suspicion of bronchiolitis and had a more stringent asthma diagnosis, including an adjudicating committee ascertaining cases if unclear. We found a lack of treatment response in asthmatic children with diagnosed RSV compared to children without diagnosed pathogen. hMPV-positive children (10% of the study population) also presented as moderate exacerbation and showed AR

for treatment failure similar to that of RSV, not reaching statistical significance with a RD for treatment failure of 8.0%.

Children who tested positive for INF (n=24) had a high risk of treatment failure (AR 37.5%). Previous studies have shown that influenza accounts for excess hospitalizations for cardiopulmonary disease in asthmatic children [34]. Yet, asthma was not identified as a risk factor for influenza related hospitalization in a large meta-analysis [6]. PIV was associated with less severe exacerbation on presentation, but a higher risk of treatment failure. PIV serotype 3, known to infect the lower airways [35], is the third most common pathogen in viral induced exacerbations, causing severe infection and lower respiratory tract symptoms in asthmatics [36]. Our total number of specimens positive for PIV was only 14, with 10 cases of PIV-3. In our 2011-2013 cohort, EV-D68 was not associated with more severe exacerbations, or with treatment failure. It is unclear if changes in subtypes may have contributed to the more recent outbreaks of severe respiratory infections associated with EV-D68 [5].

We found less infection with atypical bacteria than expected, compared to previous studies where the prevalence was 4.5% to 12.4% for *Mycoplasma pneumoniae* and 1.6% to 5% for *Chlamydophila pneumoniae* [8, 37] in children with asthma exacerbation. The use of nasopharyngeal or nasal swabs instead of throat swabs might have contributed to the lower sensitivity of our tests [38].

We did not find an association between co-infection and severity or treatment failure. It is plausible that the impact of co-infection depends on the specific interaction between pathogens

and thus is pathogen dependent. We had however insufficient power to investigate the effect of pathogen specific co-infection to make any valid conclusions.

Confirming the presence of RSV or INF in respiratory specimens of children presenting to the ED with asthma exacerbation thus identifies children with a clinical and statistical significant increased risk of treatment failure. This laboratory confirmation should ideally use RT-PCR, or if a timely turn-around time is not possible, by using point-of-care tests of the newer generation (automated readers or rapid PCR) with good accuracy in the pediatric population [39]. Further studies investigating pathogen-specific treatment response to anticholinergic bronchodilators [40], anti-viral therapy, azithromycin [41] and their combinations and a critical assessment of the effect of corticosteroids should be considered in children difficult to treat. Further exploration of preventive measures should be considered in the light of the growing knowledge that RSV, as well as HRV infections that occur early in life are associated with an increased risk of inception of asthma [42, 43]. Given the risk for influenza-related complications, such as pneumonia, children with asthma are a priority group for influenza immunization. A systematic review by Cates et al. [44] did not find evidence that immunization reduced asthma exacerbations caused by influenza virus infection, but suggested benefits on quality of life scores in relation to episodes of proven influenza infection in a small number of immunized children. We did not have information about the influenza immunization status of our patients. Given the proven safety of influenza immunization in this population and the expected protective effect, strategies to improve immunization are needed, while missed opportunities for immunization should be minimized [45].

Our study has strengths. It is the largest cohort of its kind with a richness of data that made it possible to adjust for important patient characteristics [20] and addresses the inherent heterogeneity among children with asthma. We were able to test for new pathogens including HRV species, which were not investigated in the primary study and for the most common pathogens in the asthmatic population. The presentation of AR and RD with presentation of marginal effects is a methodological strength of our analysis, making interpretation more logical and allowing for easier comparisons between the different exposure groups.

Our study also has several limitations. As we excluded children with a mild exacerbation, representing the overwhelming majority of children presenting with acute asthma to an ED, our cohort is biased towards moderate and severe exacerbation, thus limiting our ability to explore the impact of specific respiratory pathogen on the full range of exacerbation severity. Because microorganisms were identified by PCR allowing for both non-replicating viruses and colonizing microorganisms to be detected, it is possible that episodes assumed to be triggered by a respiratory pathogen were actually misclassified in children simply colonized, which may lead to inaccuracy in the conclusions about the association between the presence of a pathogen and outcomes. However, this misclassification should be non-differential and bias our results towards the null. Moreover, as parents reported that most episodes started with a URTI, it is more likely than not, that the identified microorganism was associated with symptoms. As atopy was assessed primarily by parent's report on the ISAAC and their recall of allergy testing, with no systematic objective testing with serum specific IgE or allergy testing, our ability to investigate the interaction between a specific pathogen and atopy was suboptimal. Moreover, beyond allergic status, specific allergen sensitization and recent exposure to these allergens [46] were not documented. We did not find an

additive effect of the reported atopy and identification of a specific pathogen with regards to severity or response to therapy, as reported in some studies investigating seasonal asthma exacerbation outbreaks[47] and in *in vitro* studies[48]. Finally, bronchodilator treatment was severity-specific and differed between children with moderate and severe exacerbations. Although we adjusted for severity in our treatment failure models, residual confounding may have persisted.

Conclusions:

HRV, more particularly HRV-C, is the most prevalent virus in children presenting to the ED with moderate to severe asthma exacerbation. Non-HRV pathogens as a group was associated with fewer severe exacerbation but more ED treatment failure. Specifically, RSV identified in about 18% of children with asthma, followed by INF, and PIV were associated with an increased risk of treatment failure in the ED. The findings raise the possibility that RSV *per se* confers treatment resistance at any age, not only in wheezing infants aged less than 1 year. Re-visiting interventions for RSV prevention and influenza immunization thus appear crucial to reduce the risk of poor treatment response. Rapid pathogen identification in the ED and exploration of pathogen-therapy interaction to decrease the risk of treatment failure must be further explored.

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Conflict of Interest:

The authors have no conflicts of interest relevant to this article to disclose.

Figure 1. - Flow chart of patient selection from the DOORWAY study.
(ED=emergency department)

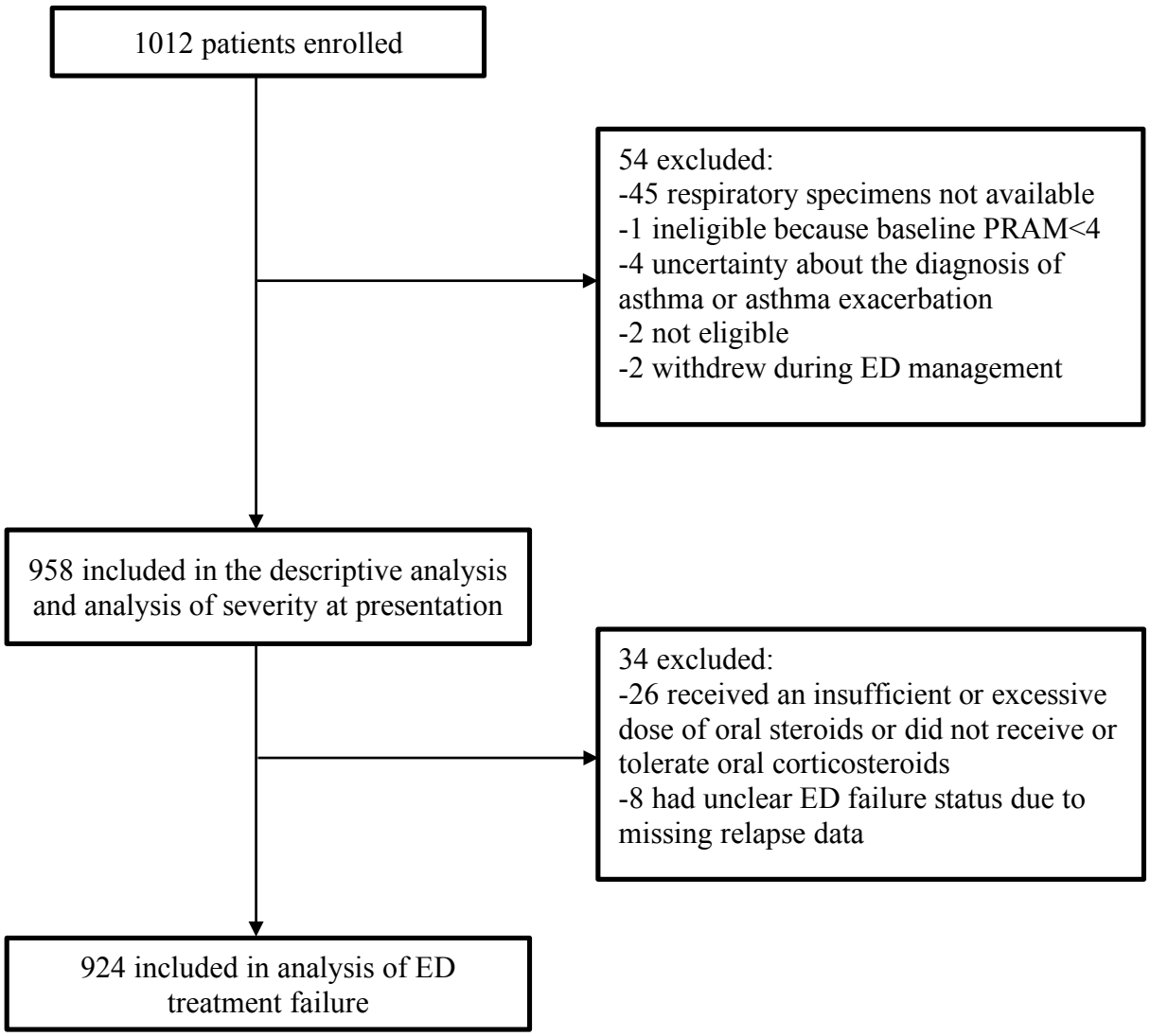


Table 1. Results of Multiplex Polymerase Chain Reaction Testing of Nasopharyngeal Aspirate Specimens

Respiratory Pathogen Identified	n (%) (N=958)
Respiratory Pathogen* Identified	591 (61.7)
Virus	590 (61.6)
Atypical Bacteria†	2 (0.2)
Number of pathogens#	
No pathogen	367 (38.3)
1 pathogen	510 (53.3)
2 pathogens	68 (7.1)
3 pathogens	13 (1.4)
Specific pathogens	
Enterovirus/Rhinovirus	
HRV	282 (29.4)
HRV-A	97 (10.1)
HRV-B	11 (1.2)
HRV-C	174 (18.2)
EV	
EV-A	1 (0.1)
EV-B	2 (0.2)
EV-C	3 (0.3)
EV-D68	25 (2.6)
Non-typed EV/HRV	14 (1.5)
RSV	171 (17.9)
RSV-A	126 (13.2)
RSV-B	46 (4.8)
hMPV	96 (10.0)
hMPV-A	46 (4.8)
hMPV-B	53 (5.5)
Influenza virus	24 (2.5)
INF-A	17 (1.8)
INF-B	7 (0.7)
ADV	12 (1.3)
ADV-A	2 (0.2)
ADV-B	0 (0)
ADV-C	10 (1.0)
ADV-E	0 (0)
PIV	14 (1.5)
PIV-1	0
PIV-2	0
PIV-3	10 (1.0)
PIV-4	4 (0.4)
Coronavirus	33 (3.4)
CoV-OC43	16 (1.7)
CoV-HKU1	6 (0.6)
CoV-229E	3 (0.3)
CoV-NL63	10 (1.0)

*Viral pathogen or atypical bacteria; †: Total of 952 specimens; #: Viral and atypical bacterial

Table 2 - Baseline Patient Characteristics. Overall and by pathogen (N,(%))

Variable	Total patients (n=958)†	Respiratory Pathogen Positive (n=591)*	Respiratory Pathogen Negative (n=367)	HRV Postive (n=282)				Non-HRV Positive (n=309)						Co-infection	
				Total	HRV-A (n=97)	HRV-B (n=11)	HRV-C (n=174)	Total	EV-D68 (n=25)	RSV (n=171)	HMPV (n=96)	INF (n=24)	PIV (n=14)	Single Pathogen Positive (n=510)	Co-infection (n=81)
Age (n=957, missing 0.1%)															
Median (IQR)	3 (2-6)	2 (1-5)	4 (2-7)	3 (1-5)	3 (2-5)	8 (2-12)	2 (1-5)	2 (1-4)	3(1-5)	2 (1-3)	2.5(1-5)	3.5(2-7)	2 (1-2)	2 (1-5)	2 (1-4)
Sex															
Male	635 (66.3)	393 (66.5)	242 (65.9)	197 (69.9)	73 (75.3)	5 (45.5)	119 (68.4)	196 (63.4)	15(60)	111 (64.91)	61(63.5)	14(58.3)	9 (64.3)	331 (64.9)	62 (76.54)
Risk Factors For Exacerbation															
Atopy	666/957 (69.6) (0.1% missing)	413 (69.9)	253 (69.1)	189 (67.0)	64 (66.0)	7 (63.6)	118 (67.8)	224 (72.5)	19(76.0)	123 (71.9)	68(70.8)	13(54.2)	9 (64.3)	366 (71.8)	47 (58.0)
Tobacco exposure	(missing 0.3%)														
cotinine level >=4 ng/ml	54/955 (5.7)	40 (6.8)	14 (3.8)	20 (7.1)	4 (4.2)	1(9.1)	15 (8.7)	20 (6.5)	0(0)	18 (10.53)	4(4.2)	2(8.3)	0 (0)	35 (6.9)	5 (6.2)
cotinine level 1-4 ng/ml	252/955(26.4)	156 (26.5)	96 (26.2)	67 (23.9)	29 (30.2)	1(9.1)	37 (21.4)	89 (28.9)	8(33.3)	38 (22.22)	27(28.1)	9(37.5)	2 (14.3)	137 (27.0)	19 (23.5)
cotinine level <1 ng/ml d	649/955 (68.0)	392 (66.7)	257 (70.0)	193 (68.9)	63 (65.6)	9 (81.8)	121 (69.9)	199 (64.6)	16(66.7)	115 (67.25)	65(67.7)	13(54.2)	12 (85.7)	335 (66)	57 (70.4)
Asthma phenotype(n=957, missing 0.1%)															
Intermittent asthma	731 (76.4)	450 (76.4)	281 (76.8)	221 (78.14)	71 (73.2)	7 (63.6)	143 (82.2)	229 (74.1)	19(76.0)	130 (76.0)	69(71.9)	18(75.0)	10 (71.4)	393 (77.1)	57 (70.4)
Persistant asthma	226 (23.6)	141 (23.9)	85 (23.2)	61 (21.6)	26 (26.8)	4 (36.4)	31 (17.8)	80 (25.9)	6(24.0)	41 (24.0)	27(28.1)	6(25.0)	4 (28.6)	117 (22.9)	24 (29.6)
Morbidity prior to Enrollment															
Patients with >= 1 course of oral corticosteroids in previous year	475/952 (46.9) (missing 0.6%)	275(46.9)	192(52.6)	140(49.7)	49(50.5)	3(27.3)	88(50.6)	135(44.3)	9(36.0)	73(43.2)	41(43.1)	9(37.5)	10 (71.4)	243(48.0)	32(39.5)
Patient with >= 1 hospital admission in previous year	146/957 (15.3) (missing 0.1%)	96(16.2)	50(13.7)	48(17.0)	16(16.5)	0(0)	32(18.4)	48(15.5)	4(16.0)	24(14.04)	8(8.3)	5(20.8)	4 (28.6)	87(17.1)	9(11.1)
Asthma quiz for kids score (median (IQR)	3(2-4)(n=938, missing 2.1%)	3(2-4)	3(2-4.5)	3(2-4)	4(2-5)	3(2-5)	3(1-4)	4(2-5)	3(1.5-3.5)	4(2-4)	4(3-5)	4(2-5)	4 (2-5)	3(2-5)	3.5(2-4)
Prescribed Asthma Controller															
None	403(42.1)	254(43.0)	149(40.6)	122(43.7)	40(41.2)	6(54.6)	76(43.7)	132(40.6)	14(56.0)	85(49.7)	36(37.5)	8(33.3)	4 (28.6)	215(42.2)	39(48.1)
Daily maintenance	279(29.1)	175(29.6)	104(28.3)	84(29.8)	29(30.0)	0(0)	55(31.6)	91(29.5)	5(20.0)	44(25.7)	30(31.3)	11(45.8)	6 (42.9)	151(29.6)	24 (29.6)
Episodic intake (when sick)	276(28.8)	162(27.4)	114(31.1)	76(27.0)	28(28.9)	5(45.5)	43(24.7)	86(27.8)	6(24.0)	42(24.6)	30(31.3)	5(20.8)	4 (28.6)	144(28.2)	18 (22.2)
Season															
Spring	225 (23.5)	128 (21.7)	97 (26.4)	79 (28.0)	47 (48.5)	1 (9.1)	31 (17.8)	49 (15.9)	0(0)	25 (14.6)	23(24.0)	5(20.8)	3 (21.4)	108 (21.2)	20 (24.7)
Summer	85 (8.9)	61 (10.3)	24 (6.5)	39 (13.8)	12 (12.4)	1 (9.1)	26 (14.9)	22 (7.1)	8(32.0)	11 (6.4)	13(5)	0(0)	1 (7.1)	50 (9.8)	11(13.6)
Fall	369 (38.5)	201 (34.0)	168 (45.8)	124 (44.0)	28 (28.9)	9 (81.8)	87 (50.0)	77 (24.9)	17(68.0)	33 (19.3)	18(18.8)	3(12.5)	5 (35.7)	186 (36.5)	15 (18.5)
Winter	279 (29.1)	201 (34.0)	78 (21.3)	40 (14.2)	10 (10.31)	0 (0)	30 (17.2)	161 (52.1)	0(0)	102 (59.7)	42(43.8)	16(66.7)	5 (35.7)	166 (32.6)	35 (43.2)
Symptoms of Infection															
Fever present	260/949 (27.4) (missing 0.9%)	172 (29.4)	88 (24.2)	45 (16.2)	15 (15.6)	1 (9.1)	29 (17.0)	127 (41.2)	4(16.7)	76 (44.7)	41(43.1)	13(54.2)	1 (7.1)	141 (27.9)	31 (38.8)
Pneumonia	87/957 (9.1) (missing 0.1%)	63 (10.7)	24 (6.5)	14 (5.0)	3 (3.1)	2 (18.2)	9 (5.2)	49 (15.9)	2(8.0)	33 (19.3)	16(16.7)	3(12.5)	0 (0)	53 (10.4)	10 (12.4)
URTI symptoms	178/957 (18.6) (0.1% missing)	116 (19.7)	62 (16.9)	61 (21.7)	19 (19.8)	3 (27.3)	39 (22.4)	55 (17.8)	3(12.0)	35 (20.5)	15(15.6)	5(20.8)	5 (35.7)	102(20.0)	14(17.3)
Study Site															
1	281 (29.3)	182(30.8)	99 (27.0)	104(36.9)	32(32.9)	1(9.1)	71(40.8)	78(25.2)	9(36.0)	45(26.3)	17(17.7)	7(29.2)	3 (21.4)	162(31.8)	20 (24.7)
2	174 (18.2)	77 (13.0)	97 (26.4)	41(14.5)	8(8.3)	1(9.1)	32(18.4)	36(11.7)	3(12.0)	16(9.4)	7(7.3)	3(12.5)	3 (21.4)	74(14.5)	3(3.7)
3	77 (8.0)	41 (6.9)	36 (9.81)	21(7.5)	9(9.3)	3(27.3)	9(5.2)	20(6.5)	2(8.0)	11(6.4)	6(6.3)	0(0)	0 (0)	38(7.5)	3(3.7)
4	426 (44.5)	291 (49.2)	135 (36.8)	116(41.1)	48(49.5)	6(54.6)	62(35.6)	175(56.6)	11(44.0)	99(57.9)	66(68.8)	14(58.3)	8 (57.1)	236(46.3)	55(67.9)
PRAM-score on presentation															
PRAM-score 4-8 (moderate)	639 (66.7)	387 (65.5)	252 (68.7)	178 (63.1)	53 (54.6)	7 (63.6)	118 (67.8)	209 (67.6)	16(64.0)	108 (63.2)	64(66.7)	14(58.3)	13 (92.9)	339 (66.5)	48 (59.3)
PRAM score 8-12 (severe)	319 (33.3)	204 (34.5)	115 (31.3)	104 (36.9)	44 (45.4)	4 (36.4)	56 (32.2)	100 (32.4)	9(36.0)	63 (36.8)	32(33.3)	10(41.7)	1 (7.1)	171 (33.5)	33 (40.7)

†Unless otherwise stated *Positive for at least 1 of the by RT-PCR investigated viral pathogens or Mycoplasma pneumoniae positive

Figure 2. - Seasonal distribution of all 25 respiratory viruses identified in 958 specimens, representing species for HRV and EV-D68 and aggregated groups for the remainder (total 10 exposures); 80 specimens were positive for more than one respiratory virus.

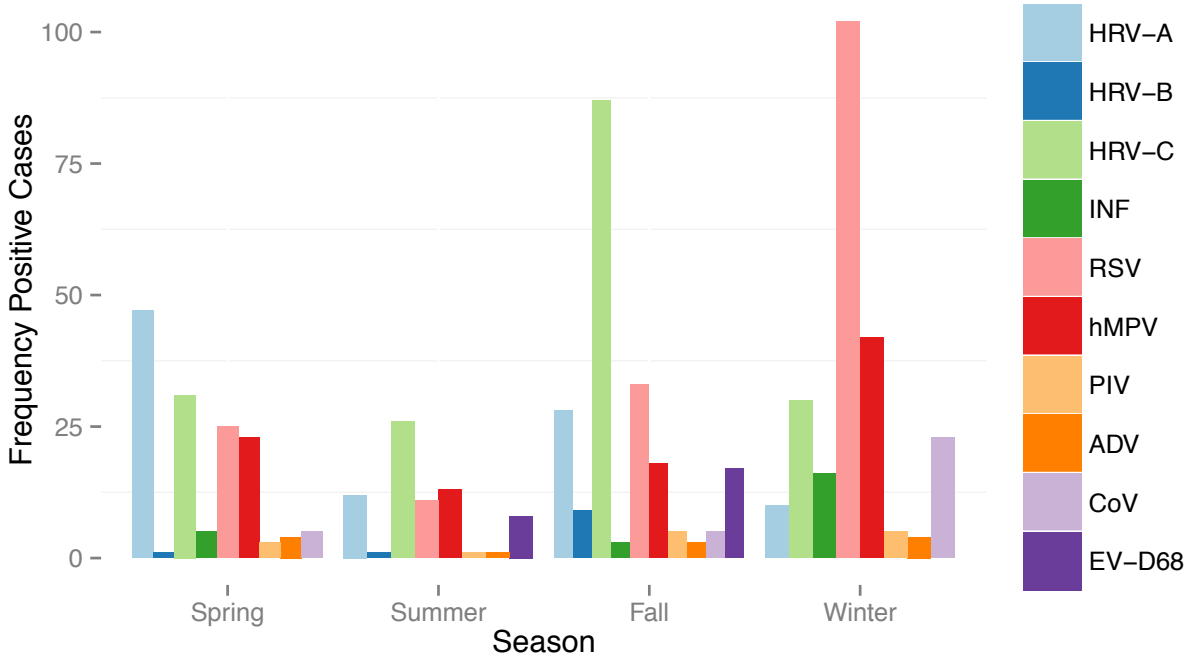
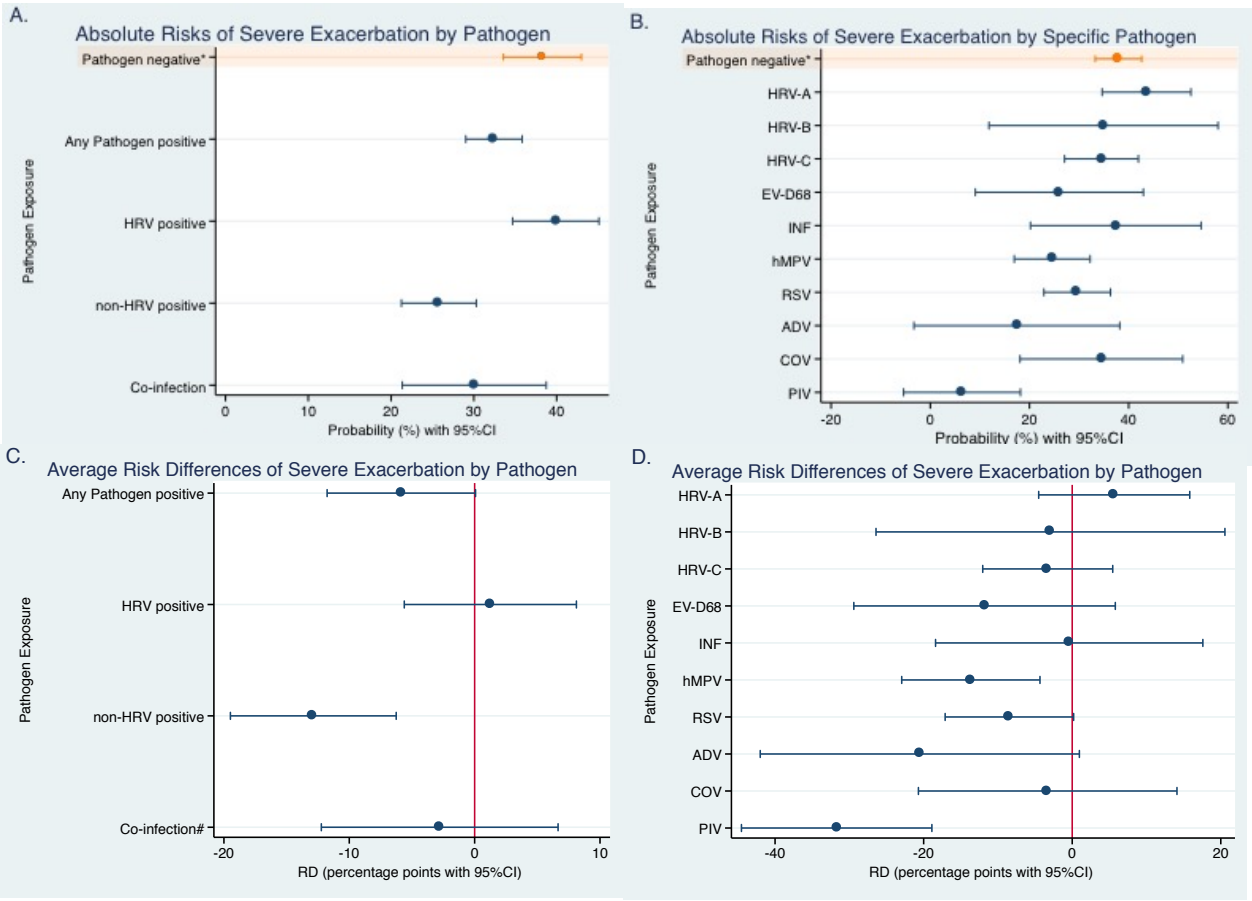


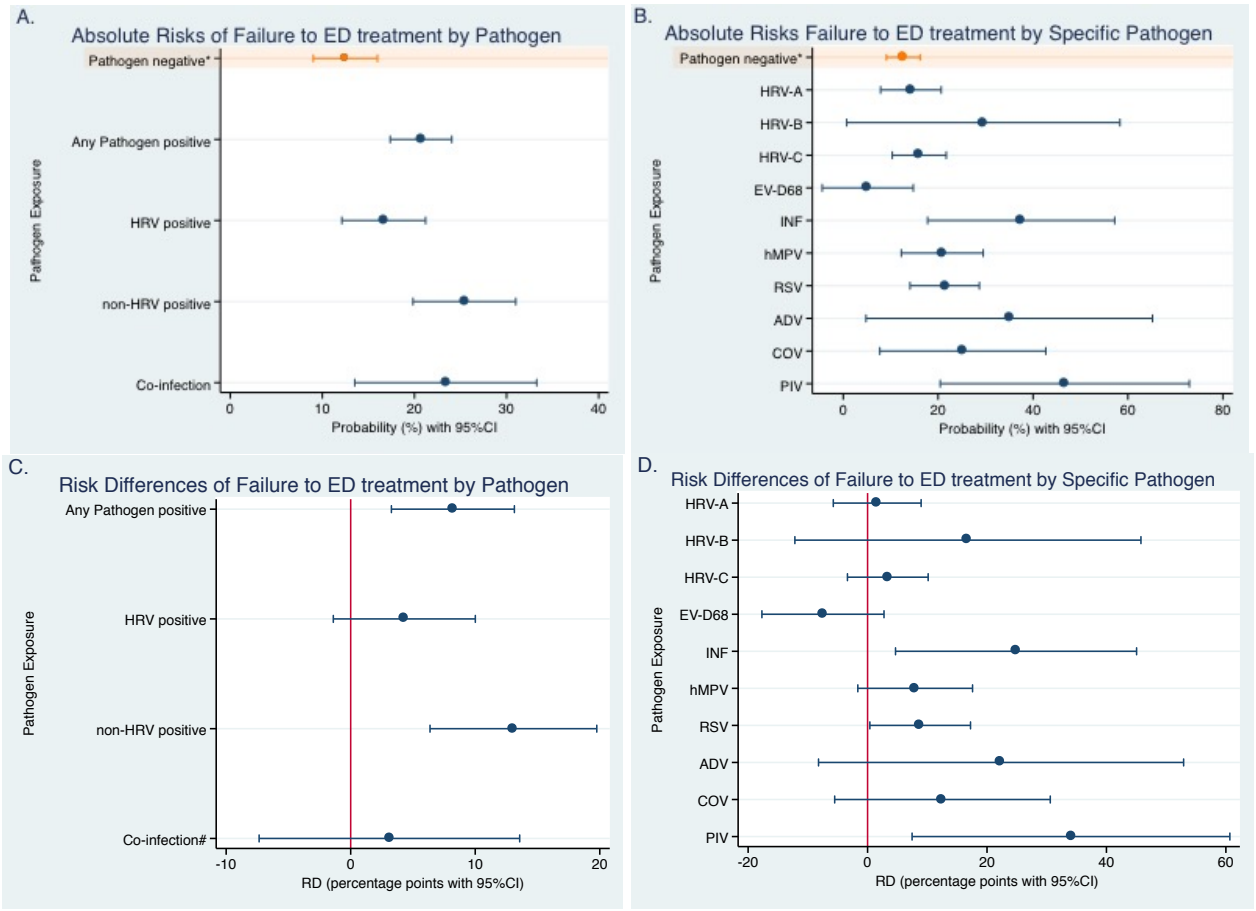
Figure 3. – Association between respiratory pathogens and the severity of exacerbation at presentation.



Average marginal effects presenting adjusted absolute risks (A and B) and adjusted risk differences (C and D) of severe exacerbation from multivariate logistic regressions. Reference used for risk differences was the pathogen-negative category for each given model (*), except for co-infection where the reference used was positive with a single pathogen (#).

ADV: adenovirus, co-infection: presence of 2 or more pathogens, CoV: coronavirus, EV-D68: enterovirus D68; hMPV: human metapneumovirus, HRV: human rhinovirus, INF: influenza virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus.

Figure 4. – Association between respiratory pathogens and ED treatment failure.



Average marginal effects presenting adjusted absolute risks (A and B) and adjusted risk differences (C and D) of emergency department treatment failure from multivariate logistic regressions. Reference used for risk differences was the pathogen-negative category (*) for each given model, except for co-infection where the reference used was positive with a single pathogen (#).

ADV: adenovirus, co-infection: presence of 2 or more pathogens, CoV: coronavirus, EV-D68: enterovirus D68; hMPV: human metapneumovirus, HRV: human rhinovirus, INF: influenza virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus.

Appendix 1 - Adjusted Absolute Risks and Risk Differences of Severe Excacerbation and Treatment Failure

Exposure	Exacerbation severity			ED Treatment Failure		
	Number of Patients Included (N)	Absolute Risk % (95%CI)	RD % (95%CI)	Number of Patients Included (N)	Absolute Risk % (95%CI)	RD % (95%CI)
Total Population (crude)						
Total Population	958	33.3(30.3; 36.3)		924	16.9 (14.5; 19.3)	
Respiratory Pathogen in general†						
Pathogen negative	840	38.3(33.6; 43.0)	ref.	803	12.5(9.0; 16.0)	ref.
Pathogen positive	840	32.4(29.0; 35.8)	-5.8(-11.8; 0.01)	803	20.7 (17.4; 24.1)	8.2 (3.3; 13.1)
HRV positive versus non-HRV positive versus Pathogen negative‡						
Pathogen negative	840	38.6(34.1;43.2)	ref.	803	12.4 (8.9; 15.8)	ref.
Non-HRV positive	840	25.8(21.3; 30.3)	-12.9(-19.5;-6.3)	803	25.4 (19.8; 31.0)	13.1 (6.4; 19.8)
HRV-positive	840	39.9 (34.7;45.1)	1.3(-5.6-8.1)	803	16.7 (12.1; 21.2)	4.3 (-1.4; 10.0)
Pathogen Specific*						
Pathogen negative	840	37.9 (33.3; 42.6)	ref.	808	12.7 (9.1; 16.2)	ref.
HRV-A	840	43.6 (34.6; 52.6)	5.7 (-4.5; 15.8)	808	14.3 (7.9; 20.7)	1.6 (-5.7; 9.0)
HRV-B	840	34.9(11.8;58.0)	-2.9 (-26.4; 20.6)	808	29.5 (0.7; 58.3)	16.8 (-12.2; 45.8)
HRV-C	834	34.5(27.1; 42.0)	-3.3 (-12.0; 5.5)	808	16.0 (10.3; 21.7)	3.4 (-3.4; 10.1)
EV_D68	840	26.0(9.1; 43.0)	-11.8 (-29.4; 5.8)	808	5.2 (-4.4; 14.8)	-7.5 (-17.7; 2.8)
INF	840	37.4(20.2; 54.6)	-0.4 (-18.4; 17.6)	808	37.5 (17.8; 57.2)	24.9 (4.7; 45.1)
HMPV	840	24.6(16.9; 32.2)	-13.6 (-23.0; -4.3)	808	20.9 (12.2; 29.5)	8.0 (-1.6; 17.6)
RSV	840	29.6(22.9; 36.3)	-8.5(-17.1; 0.2)	803	21.4 (14.1; 28.7)	8.8 (0.4; 17.2)
ADV	840	17.5(-3.3; 38.2)	-20.5 (-42.0; 1.0)	808	35.0 (4.8; 65.2)	22.4 (-8.2; 52.9)
COV	840	34.5(18.1; 50.9)	-3.3 (-20.7; 14.1)	808	25.2 (7.7; 42.7)	12.5 (-5.5; 30.6)
PIV	840	6.4(-5.3; 18.2)	-31.7 (-44.5; -18.9)	808	46.7 (20.4; 73.0)	34.1 (7.5; 60.7)
Co-infection#						
Single pathogen positive	840	32.8 (29.1; 36.6)	ref.	803	20.3 (16.7; 23.8)	ref.
Co-infection	840	30.0 (21.3; 38.7)	-2.8(-12.2; 6.7)	803	23.4 (13.5; 33.3)	3.1 (-7.4; 13.6)

†Presence of a viral pathogen or *Mycoplasma pneumoniae*; Model for severity: interaction of exposure with asthma phenotype and income group, adjusted for age, sex, atopy, prior steroid use, asthma control, control medication, season, tobacco exposure, site;
Model for treatment failure: interaction of exposure with fever, adjusted for age, sex, PRAM score, asthma phenotype, prior steroid use, asthma control, control medication, season, tobacco exposure, atopy, income group, site
‡Model for severity: adjusted for age, sex, asthma phenotype, prior steroid use, asthma control, control medication, season, tobacco exposure, atopy, income group, site; Model

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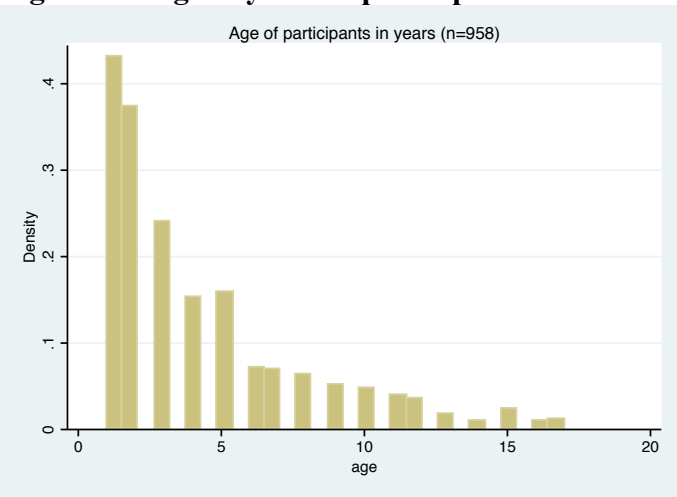
III. Extra Results Section

1. Descriptive analysis

-Population characteristics:

Figure 3. - Age in years of participants.



- Lacking data on Z-score BMI in 406/958 patients.

- The 92 patients that had missing value for income had the same exposure- outcome distribution as the general population.

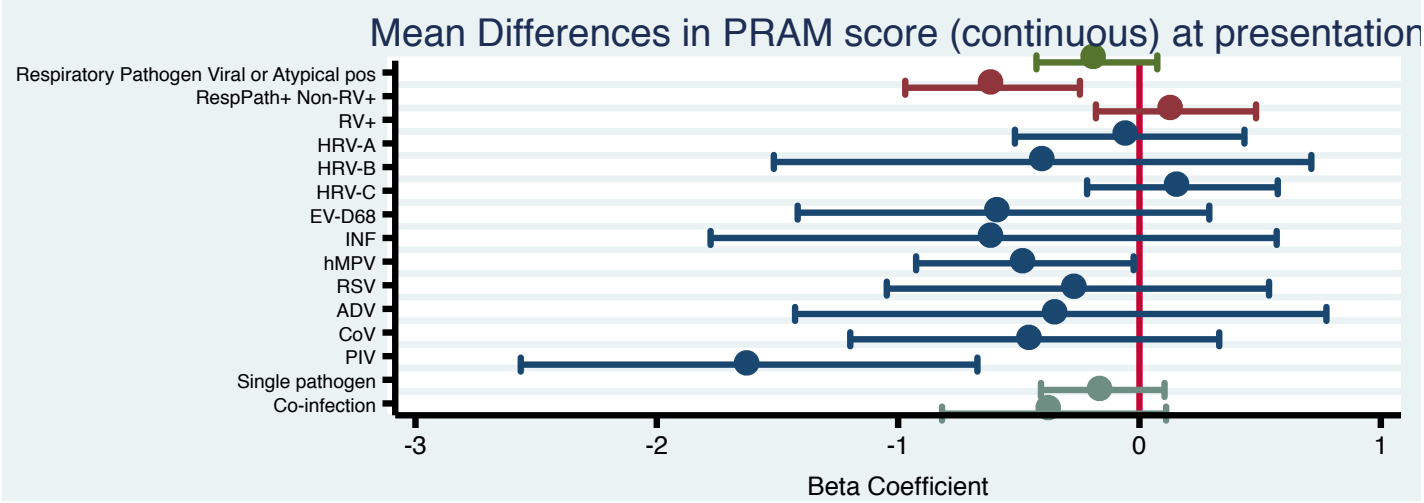
Figure 4. - Average neighbourhood financial income per study site included patients.

-The MD was asked to identify the main trigger of the exacerbation which was summarized as infectious versus non infectious. Using a positive diagnosis of a pathogen on the respiratory specimen as the reference, the MD judgement had a sensitivity of 93.5% and a specificity of 9.3% for the presence of a pathogen.

2. Outcome exacerbation severity

- PRAM-score as a continuous outcome.

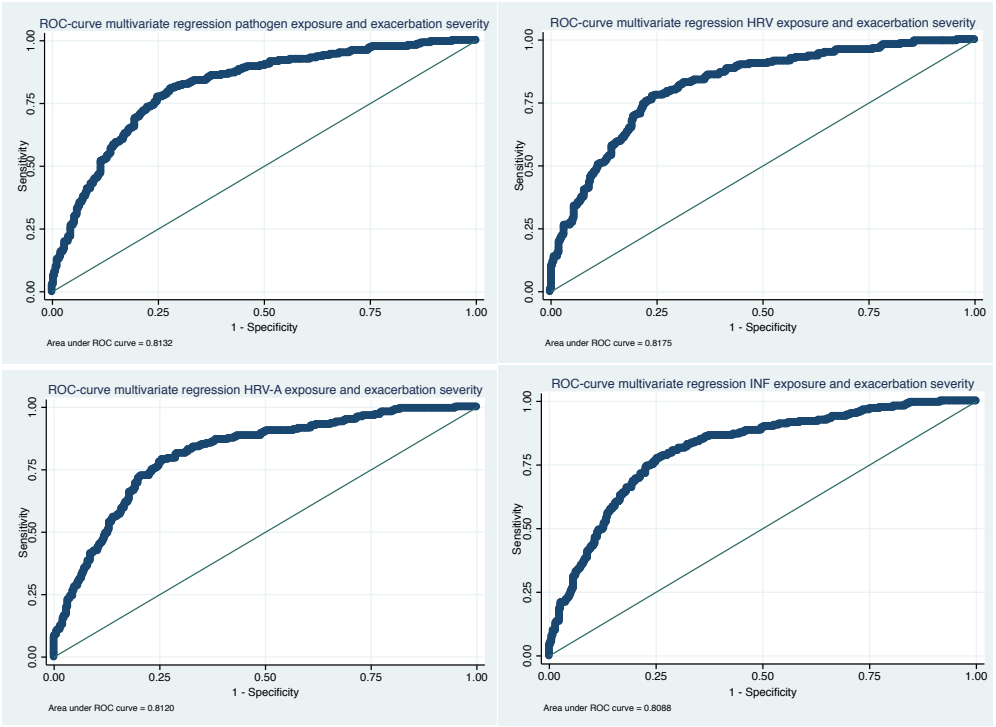
Figure 5. - Coefficient plot presenting beta-coefficients of exposures used in models investigating the association of respiratory pathogens and the continuous outcome PRAM-score



Legend:
-Model exposure respiratory pathogen viral of atypical pos: Presence of a viral pathogen or *Mycoplasma pneumoniae*: adjusted for age, sex, atopy, prior steroid use, asthma control, control medication, asthma phenotype, season, tobacco exposure, site, income group;
-Model respPath+ Non-RV+ and RV+: Rhinovirus positive vs non-HRV pathogen positive vs negative: interaction with fever; adjusted for age, sex, asthma phenotype, prior steroid use, asthma control, control medication, season, tobacco exposure, atopy, income group, site;
-Models pathogen specific from HRV-A up to and including PIV: Separate models per specific pathogen exposure: interaction of HRV-A with URTI; interaction of HRV-C with fever and pneumonia; interaction of EV-D68 and fever; interaction of INF and fever; interaction of RSV and income; adjusted for co-infection, age, sex, asthma phenotype, prior steroid use, asthma control, control medication, season, tobacco exposure, atopy, income group, site;
-Model Co-infection: Presence of ≥ 2 pathogens vs. no pathogen and a single pathogen vs. no pathogen; adjusted for age, sex, atopy, asthma phenotype, prior steroid use, asthma control, control medication, season, tobacco exposure, income group, site; model for treatment failure: interaction co-infection with fever

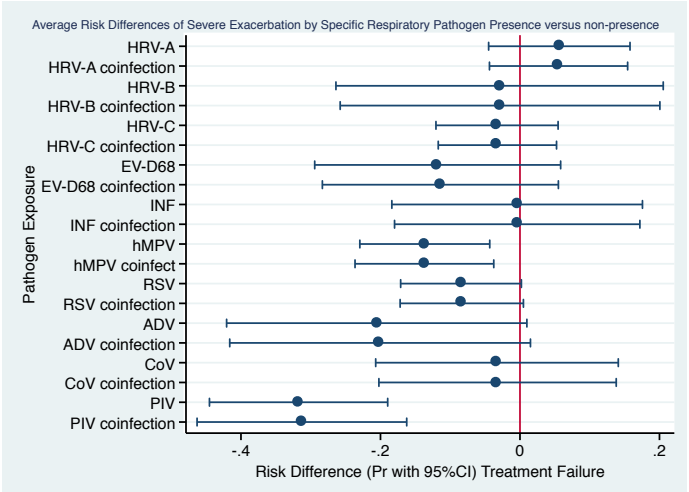
-Using PRAM score as a binary outcome: moderate or severe exacerbation.

Figure 6. - Area Under the Curve (AUC) presented as ROC for the outcome moderate vs. severe exacerbation



Legend: AUC values are graded as: 0.90–1.0: excellent discrimination; 0.80–0.90: good discrimination; 0.70–0.80: fair discrimination; 0.60–0.70: poor discrimination; 0.50–0.60: failed discrimination

Figure 7. - Risk differences of exposures used in models investigating the association of respiratory pathogen and binary outcome treatment failure, comparing RDs stratified by co-infection not present vs. co-infection present. Co-infection was used as a binary variable: co-infection vs. single or no pathogen diagnosed.



3. Outcome treatment failure

Figure 8. - Area Under the Curve (AUC) presented as ROC for the outcome treatment failure.

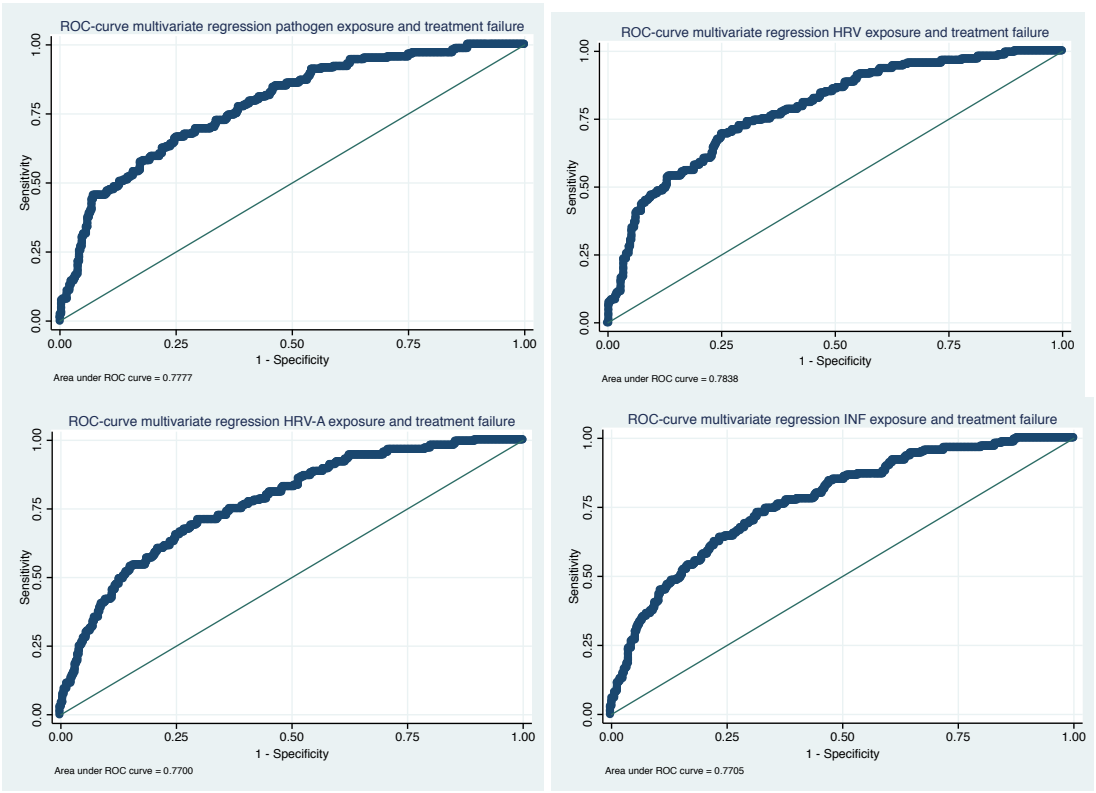
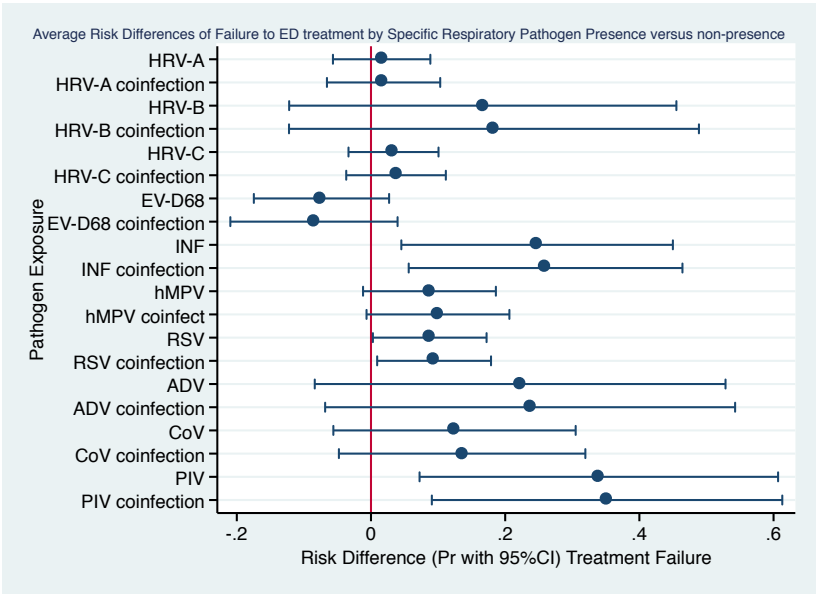


Figure 9. - : Risk differences of exposures used in models investigating the association of respiratory-specific pathogen and ED treatment failure, comparing RDs stratified by co-infection not –present and co-infection present. Co-infection was used as a binary variable: co-infection vs. single or no pathogen diagnosed.



Chapter 6: A final discussion and summary

Restatement of objectives and findings

Respiratory pathogens are the most frequent triggers of asthma exacerbations in children. Yet, little is known about the potential clinical impact of these laboratory-diagnosed pathogens on the severity of the exacerbation, as well as on the response to the standardized ED treatment with systemic corticosteroids and inhaled bronchodilators. In this thesis, I first summarized the peer-reviewed published literature in regard to the association between the presence of respiratory pathogens and clinical outcomes, namely: asthma exacerbation severity, health care utilization, treatment response, and other indicators of morbidity and mortality. Only a limited number of studies had investigated the associations of interest and the quality of the evidence was low in general. There was possible evidence for higher severity of asthma exacerbation on presentation in children presenting to the ED, in the presence of HRV, and more specifically of HRV-C. Evidence was lacking, however, regarding any association between respiratory pathogens in general or with specific pathogens with regard to health care utilization or other indicators of morbidity. Moreover, potentially clinically important associations, including interaction with atopy were insufficiently investigated. There were only a limited number of well-powered studies for pathogen-specific investigation. The large prospective cohort study of Ducharme et al, on children, 1 to 17 years old, presenting to the ED with moderate or severe exacerbations over 3 consecutive years, was included in our systematic search. The DOORWAY study showed an association between the presence of a pathogen in general and ED treatment failure consisting of treatment with corticosteroids and severity-specific bronchodilators.

For the second manuscript, I used the data from the DOORWAY study for a secondary analysis. I first refined pathogen identification by using sequencing on patients' respiratory specimens and confirmed that HRV was the most prevalent laboratory-diagnosed pathogen, with a majority of HRV-C, in children presenting to the ED with an exacerbation. Additionally, I tested the specimens for the presence of atypical bacteria. I investigated the association between a laboratory-diagnosed pathogen (overall and specific) and both the severity of the exacerbation on presentation to the ED and treatment response, investigating the risk of treatment failure. No specific pathogen was associated with increased severity. However, children presenting with hMPV and PIV had a lower risk for severe exacerbation than children presenting in the absence of a pathogen. Children diagnosed with RSV, INF and PIV were at increased risk of treatment failure, needing hospitalization, prolonged ED stay or re-admission compared to children without diagnosed pathogen, suggesting a differential pathogen-specific treatment response.

Strengths and Limitations

In this thesis, I summarized the available literature on the association between respiratory pathogens and clinical relevant outcomes of asthma exacerbation by conducting a systematic review. This method of reviewing the peer-reviewed published literature is a highly valued manner to summarize and synthesize the state of the art on an investigated topic (98). Hereafter, I did a secondary analysis of the large prospective cohort study of Ducharme et al, the largest study of its kinds with a rich dataset, which permitted pathogen-specific investigation of different associations. We additionally tested the positive enterovirus-rhinovirus specimens for further rhino-enterovirus identification, which allowed us to confirm the high prevalence of the HRV-C species in children with asthma presenting to the ED.

Because our study question was answered using previously collected data, *a priori* sample size calculation was however not possible, nor could be decided on the collection of additional co-variates. Our study population was limited to children presenting to the ED with an exacerbation of at least moderate severity. Selection bias might be introduced, by only investigating children with an exacerbation severe enough to seek health care and disregarding those with mild exacerbation presenting to the ED. In addition, might prior exacerbations and socio-economic characteristics influence which children present at the ED in a differential way.

In this thesis, I can only conclude on possible associations between specific pathogens and the investigated clinical outcomes. Causal methods would be preferred to investigate the impact of the pathogens on the clinical relevant outcomes. However, the presence of a pathogen in a respiratory specimen, diagnosed with PCR, does not guarantee its causal implication in the exacerbation. Furthermore, episodes could have been misclassified as triggered by a certain pathogen, while non-replicating virus is present.

Multiple imputation for missing data and a laboratory based definition of atopy could have possibly improved the quality of my analysis. Our ability to investigate the interaction between a specific pathogen and atopy was suboptimal, both in the reviewed literature and in our study analysis, due to insufficient reporting and measurement, respectively. And last but not least, residual confounding by unmeasured and undefined confounders can maintain present.

Future Research

Our findings will need replication. Further research should use comparable exposure, clear, stringent and similar covariates and outcome definitions, rigorous statistical methodology and importantly, focus on pathogen-specific treatment response investigating patient oriented and clinical outcomes.

This is in line with a recent European consensus (99) pertaining to the 15 top asthma research priorities, which included the investigation of the impact of micro-organisms on asthma in general, and asthma exacerbations, specifically. The impact, feasibility and costs of timely and accurate diagnosis of respiratory pathogens in children presenting with an asthma exacerbation by the use of multiplex PCR or by rapid testing at the point-of-care of a subset of key pathogens should be investigated. Vigilance for new and emerging pathogens and mutations/changes in current circulating species that could have an impact on the clinical presentation of children with an asthma exacerbation is warranted. Children with asthma are an important subgroup for stratification and vigilance within surveillance data. Additionally, further investment should go towards improvement of preventive measurements for respiratory infection, with the development of a better vaccine against influenza and further investigation of RSV vaccines, importantly looking at their impact on hospitalization. Last but not least, studies should look at the role, feasibility and impact of pathogen-specific treatment intensification options in this population at risk.

Summary

HRV, with species HRV-C is the most prevalent pathogen in children presenting to the ED with moderate to severe asthma exacerbation. While the literature reports some evidence of increased exacerbation severity on presentation to the health-care setting associated with the presence of HRV-C, we could not find a specific pathogen positively associated with severity of exacerbation on presentation in children with moderate or severe asthma exacerbation presenting to the ED in our large cohort. With regards to treatment response, RSV, INF and PIV were associated with higher risk of ED management failure in response to oral corticosteroids and severity-specific

bronchodilators, increasing the absolute risk of treatment failure from 16.9% in the total study population up to 21.4% for children with laboratory diagnosed RSV and 37.5% for children with diagnosed influenza. This supports the need for further exploration of the need for RSV prevention and influenza immunization; timely pathogen diagnosis at presentation and exploration of pathogen-therapy interaction and follow-up regimens in children at risk for difficult to treat asthma exacerbations.

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