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DISSERTATION

Key clinical features and epidemiological patterns associated with influenza and other respiratory viral infections in children-a prospective cohort study

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Barbara Rath, Tim Conrad, Puja Myles, Maren Alchikh, **Xiaolin Ma**, Christian Hoppe, Franziska Tief, Xi Chen, Patrick Obermeier, Bron Kisler, Brunhilde Schweiger. Influenza and other respiratory viruses: standardizing disease severity in surveillance and clinical trials. **Expert Review of Anti-infective Therapy**. 2017; 15(6):565-568. (**IF: 3.141**)

Xiaolin Ma, Tim Conrad, Maren Alchikh, Janine Reiche, Brunhilde Schweiger, Barbara Rath. Can we distinguish respiratory viral infections based on clinical features? A prospective pediatric cohort compared to a systematic literature review. **Reviews in Medical Virology**. 2018; 28(5):e1997 (**IF: 5.034**)

Table of Contents

2 List of Abbreviations

Ab	antibody	COH	inception cohort dataset
AE	asthma exacerbation	CPAP	continuous positive airway pressure
Altered/LOC	altered or loss of consciousness	CS	cross-sectional
ARI	acute respiratory infections	DB	difficulty breathing
AT	Argentina	DF	difficulty feeding
AU	Australia	DFA	direct immunofluorescence assay
AUC	area under the receiver operating characteristic curve	dNTP	desoxynucleoside triphosphate
BALF	bronchoalveolar lavage fluid	DTT	dithiothreolin
BCL	bronchiolitis	dUTP	desoxyuridine triphosphate
BiPAP	biphasic positive airway pressure	ECDC	European Centre for Disease Prevention and Control
BSA	bovine serum albumin	ECMO	extracorporeal membrane oxygenation
CAP	community-acquired pneumonia	EIA	enzyme immunoassay
CART	classification and regression tree	EIFA	enzyme immunofluorescence assay
CC	case-control	ER	emergency room
CDC	Centers for Disease Control and Prevention	ETA	endotracheal aspirate
CF	cystic fibrosis	FRI	febrile respiratory illness
CI	confidence interval	FS	febrile seizure
CIDT	conditional inference decision tree	GU	Guatemala

HAdV	human adenovirus	NPA	nasopharyngeal aspirate
HBoV	human bocavirus	NPS	nasopharyngeal swab
HCoV	human coronavirus	NS	nasal swab/secretion
HHP-6	human herpesvirus 6	NW	nasal washing
HMPV	human metapneumovirus	OP	observational prospective
HPIV	human parainfluenza virus	OPS	oropharyngeal swab
HRV	human rhinovirus	OR	odds ratio
ICD	international classification of diseases	ORT	observational retrospective
ICU	intensive care unit	PC	prospective cohort
IFA	indirect immunofluorescence assay	PNA	pneumonia
ILI	influenza-like illness	pOR	pooled odds ratio
IV	influenza virus	PPV	positive predictive value
LIT	literature review dataset	PS	pharyngeal swab
LRM	logistic regression methodology	QM	quality management
LRTI	lower respiratory tract infections	RKI	Robert Koch Institute
$MgCl_2$	magnesium chloride	RS	respiratory sample
M-MLV	moloney murine leukemia virus	RSV	respiratory syncytial virus
MZ	Mozambique	RT	rapid test
NAIs	neuraminidase inhibitors	RTI	respiratory tract infection
NIC	national influenza center	RT-PCR	reverse transcription polymerase chain reaction
NL	Netherlands	SARI	severe acute respiratory infection
NOPS	number of positive specimen	TA	tracheal aspirate
NOS	number of specimen	TNP	total number of patient

TS	throat swab
UK	United Kingdom
US	United States
URTI	upper respiratory tract infections
VC	virus culture
WHO	World Health Organization
ys	years

3 List of Tables

4 List of Figures

5 Abstract (in German and English)

5.1 Zusammenfassung (abstract in German)

Einleitung

Influenza- und akute Atemwegsinfektionen zählen weltweit zu den Hauptursachen für Morbidität und Mortalität bei Kindern. In der Regel sind Influenza-Symptome nicht-spezifisch und ähneln Erkrankungen anderer Atemwegserreger, insbesondere bei Kindern. Eine gesicherte klinische Diagnose ist jedoch entscheidend für die rechtzeitige und kostenwirksame Anwendung von antiviralen Medikamenten. Obwohl Laboruntersuchungen weit verbreitet sind, wird die klinische Entscheidungsfindung meist durch eine mutmaßliche klinische Diagnose beeinflusst. In dieser Studie sollten deshalb wesentliche klinische Merkmale identifiziert werden, die eine klinische Differenzierung zwischen verschiedenen Atemwegsviren erleichtern.

Methodik

Von 12/2009 bis 04/2015 wurden prospektiv Kinder mit grippe-ähnlicher Symptomatik (ILI) in die Studie aufgenommen. Proben des Nasopharyngealtraktes wurden mit Multiplex real-time RT-PCRs auf Influenzavirus (IV), respiratorisches Syncytialvirus (RSV), Adenovirus (AdV), Rhinovirus (HRV), Metapneumovirus (HMPV), Bocavirus-1 (HBoV-1), Parainfluenza-Virus und Coronavirus untersucht. Zur Ermittlung von Erreger-assoziierten Symptomen wurden auf Basis klinischer und virologischer Daten Meta-Analysen innerhalb der Studienkohorte sowie im Vergleich zu einem selbst erstellten Literaturreview durchgeführt. Weiterhin wurde der CIDT (**c**onditional **i**nference **d**ecision **t**ree) Algorithmus eingesetzt, um Entscheidungsbäume für die Ermittlung der Infektionswahrscheinlichkeit für alle in dieser Studie untersuchten Atemwegsviren zu berechnen.

Ergebnisse

Von insgesamt 6042 Proben waren 70% positiv für mindestens ein Virus. Neben HRV (22%), trugen RSV (17%), HBoV-1 (16%) und IV (11%) beträchtlich zu ILI bei Kindern bei. HMPV (4%) war der am wenigsten entdeckte Erreger. Saisonales epidemisches Zirkulationsverhalten wurde vorrangig für IV, RSV und HMPV beobachtet. Die Meta-Analyse der klinischen

Parameter zeigte eine signifikante Assoziation von Kopfschmerzen und Durchfall mit nur IV bzw. AdV; andere Parameter wie Husten, Dyspnoe, Fieber, Rhinitis oder Keuchen/untere Infektionen der Atemwege (LRTI) sind mit mehr als einem Virus assoziiert. Das IV-Vorhersagemodell, welches Fieber, Kinder jünger zwei Jahre und saisonale Zirkulation verwendet, zeigte eine Sensitivität von 69,8% und eine Spezifität von 89%; das Vorhersagemodell für RSV, das Husten, LRTI, Kinder jünger zwei Jahre und Saisonalität berücksichtigt, hatte eine Sensitivität von 69,6% und Spezifität von 83%. Die Entscheidungsbäume für die Vorhersage der anderen Erreger waren weniger sensitiv/spezifisch.

Schlussfolgerung

Ein klinisches Einzelmerkmal kann nur begrenzt für die Vorhersage einer Infektion mit einem bestimmten Atemwegsvirus angewendet werden. Das Vorhersagemodell für IV ermöglicht die Einordnung von Patienten nach der Wahrscheinlichkeit einer IV-Infektion und kann dadurch dem Kliniker bei der Entscheidung zu einem Labornachweis oder der Verschreibung eines Virostatikums helfen. Der Baum zur Ermittlung der Wahrscheinlichkeit einer RSV-Infektion kann ebenso für den Ausschluss einer Infektion mit IV verwendet werden. Weiterführende Evaluationen der aufgeführten Algorithmen sind notwendig, um die klinische Entscheidungsgrundlage für Laboruntersuchungen oder Behandlungen zu optimieren.

5.2 Abstract

Introduction

Influenza and acute respiratory infections are leading causes of childhood morbidity and mortality worldwide. Influenza symptoms are usually non-specific and similar to other respiratory viral infections, particularly in children. However, confirmed clinical diagnosis is crucial for the timely and cost-effective application of antivirals. Although laboratory testing is widely available, making a clinical decision is influenced mostly by presumptive clinical diagnosis. This study therefore aimed to identify key clinical features to facilitate clinical differentiation between different respiratory viruses.

Method

From 12/2009 to 04/2015, children with influenza-like illness (ILI) were prospectively enrolled. Nasopharyngeal specimens were investigated for influenza virus (IV), respiratory syncytial virus (RSV), adenovirus (HAdV), rhinovirus (HRV), metapneumovirus (HMPV), bocavirus-1 (HBoV-1), parainfluenza virus and coronavirus by multiplex real-time RT-PCR. For the determination of pathogen-associated symptoms, meta-analyses were performed based on clinical and virological data within the study cohort (COH) and in comparison to a self-implemented literature review. Conditional inference decision tree (CIDT) algorithms were used to develop three models for predicting each of the respiratory viruses analyzed in the COH.

Results

Of 6042 specimens, 70% were positive for at least one virus. Besides HRV (22%), RSV (17%), HBoV-1 (16%), and IV (11%) considerably contributed to ILI in children. HMPV (4%) was the least detected pathogen. Epidemic seasonal circulation was observed primarily for IV, RSV, and HMPV. Clinical feature analyses determined significant associations of headache and diarrhea to only IV and HAdV, respectively; other features, e.g. cough, dyspnea, fever, rhinitis, or wheezing/ lower respiratory tract infection (LRTI) were shared by more than one virus. The CIDT predictive model for IV which included fever, children younger than two years and seasonal pattern yielded a sensitivity of 69.8% and a specificity of 89%; the CIDT model for RSV including cough, LRTI, children younger than two years and the seasonal

pattern had a sensitivity of 69.6% and a specificity of 83%. CIDT models for other pathogens showed lower sensitivity/specificity.

Conclusion

Individual features alone provide limited value for predicting any specific type of respiratory viral infections. The predictive CIDT model, particularly for IV allows stratification of patients into risk groups and may help clinicians to decide for laboratory testing of IV and prescription of antivirals. A first CIDT model for RSV was developed which can be used to further distinguish patients at high risk for IV. Further evaluation of these algorithms will be necessary to optimize decision rules for testing and treatment.

6 Introduction

6.1 Disease burden of acute respiratory infections in children

Influenza and acute respiratory infections (ARI) are leading causes of childhood infection and disease-related morbidity, mortality and hospitalization worldwide, particularly in children younger than five years old [1-3]. Young children experience three to ten episodes of ARI a year [4, 5]. Globally, around 30%–60% of pediatric outpatient visits and 20%–30% of hospital admissions are attributed to ARI. The number of childhood deaths attributed to ARI each year is high and estimated to be 1.9 million (95% confidence interval [CI], 1.6–2.2 million) each year according to data from the World Health Organization (WHO) [1]. Viral ARI cause approximately 20% of all deaths in pre-school children worldwide [6]. However, most respiratory deaths are attributed to acute lower respiratory tract infections (LRTI), particularly pneumonia [7].

The causative agents for ARI include viruses, bacteria and fungi. ARI are predominately caused by viral pathogens in children, and respiratory viruses account for 90% of ARI and nearly 50% of cases of community-acquired pneumonia [8]. ARI are the most frequent symptomatic reason to seek or visit primary or ambulatory care, resulting in a considerable disease burden. It has been reported that more than 70% and approximately two-thirds of primary care visits do not necessitate an office visit or antibiotic management, respectively [9]. Additionally, ARI visits constitute approximately up to one-tenth of ambulatory visits, and hospitals often experience winter surges in admissions caused by ARI [9, 10]. The etiology of ARI is complex and diverse. In industrialized countries, influenza virus (IV), respiratory syncytial virus (RSV), human parainfluenza virus (HPIV), human adenovirus (HAdV), and human rhinovirus (HRV) are leading causes of ARI in children as well as adults [11]. Over the past decade, several new pathogens have been identified, including human metapneumovirus (HMPV) [12], novel types of human coronavirus (HCoV), such as NL63 [13] as well as HKU1 [14], and human bocavirus (HBoV) [15]. Among hospitalized children in Germany, the top five most frequently detected viruses included RSV, HRV, HBoV, IV and HPIV, while other respiratory viruses such as HMPV, HAdV and HCoV, have also been detected with lower frequency [16, 17]. Both IV and RSV are the leading underlying etiologies of the two most significant respiratory syndromes diagnosed in routine clinical practice, e.g., influenza-like illness (ILI) or ARI [18-20]. The European Centre for Disease Prevention and Control (ECDC)

reported that among the 31 infectious diseases monitored, influenza topped the list in disease burden (30% of the total burden) [21]. Although IV can affect populations of all ages, young children have been identified as a population susceptible to IV infections [22]. IV-related hospitalization in children has been estimated to be around 610,000–1,237,000 cases per year worldwide [23]. RSV is also one of the most frequent causes of hospitalization in children. It has been reported that RSV is related to approximately 28% of all LRTI cases and 13%–22% of all LRTI mortality in young children under 5 years of age worldwide [24]. In 2015, Shi et al. estimated that there were 21.6–50.3 million episodes of RSV-associated LRTI worldwide, which resulted in approximately 2.7–3.8 million hospitalizations and 48,000-74,500 in-hospital deaths in (670.5 million) children under 5 years of age [24].

6.2 Clinical picture and diagnosis of acute respiratory tract infection in children

ARI refer to all infections of the respiratory tract such as upper respiratory tract infections (URTI) and LRTI. Annually, children experience multiple episodes of ARI [25]. Children with ARI often present with complaints, such as fever, cough, and tachypnea, while nasal flaring, grunting, chest retractions, cyanosis, rales and rhonchi can also be found by clinical examination [26].

URTI include rhinitis, pharyngitis/tonsillopharyntitis/laryngtitis–often called the common cold–sinusitis, and acute ear infections. There are several commonly presented symptoms of URTI, such as cough, sore throat, running nose, nasal congestion, headache low-grade fever and sneezing. Viral pathogens account for the majority of URTI etiologies. HRV, HAdV, RSV, HMPV and IV are the most commonly detected viruses in children [27 5, 28]. HRV constitutes around 25%–34% of URTI, followed by HAdV constituting 13%–25%, RSV, HPIV, IV and HMPV accounting for 25%–35%, HCoV accounting for around 10%, and the unidentified viruses accounting for the remaining [27 5, 28]. However, there are subtle differences regarding the age-related prevalence of these viruses in children. For instance, among children aged both under 2 years of age and 5-9 years of age, HRV is the most commonly detected virus, followed by HAdV. HRV is the most commonly detected in children aged between 2–4 years old, and IV is the predominate virus in children aged 10–17 [28]. LRTI is defined as any infection affecting the trachea, airways or lungs, and often refers to pneumonia, acute

bronchitis and bronchiolitis. Symptoms of LRTI vary with regard to different types of infection, but often include fever, cough, wheezing, shortness of breath, fatigue and lethargy. Currently, RSV, HRV, HAdV and HBoV are recognized as the most common viral causes LRTI, particularly for pneumonia [29-32]. Other respiratory viruses such as HMPV, IV, HCoV and HPIV are also detected [29-32]. Several studies have been conducted to validate the clinical signs and symptoms to diagnose IV. However, in general, poor sensitivity and specificity are revealed. Previous evidence has suggested that both the overall sensitivity and positive predictive value (PPV) of a "clinical" IV diagnosis based on clinical examination and patient history in children is below 40% [33]. The signs and symptoms of infection are usually non-specific, especially in children, which renders it difficult to differentiate from infections caused by other respiratory viruses. The ability to clinically and accurately diagnose IV infection is further hindered by the common succession of different respiratory infections in the winter months [33].

The diagnostic codes of International Classification of Diseases (ICD) and laboratory reports are the two main approaches for hospital-based estimates of ARI [34-36]. However, the application of ICD-codes varies in clinical practice. For instance, several codes are applied to make a diagnosis, while others are used to demonstrate a clinical manifestation or suspicion [35, 37, 38]. The signs and symptoms of respiratory viral infections are atypical, especially in children, and as a consequence, children might receive a diagnosis of ICD-codes in agreement with non-respiratory symptoms, such as febrile seizures and gastroenteritis [39, 40]. In addition, the practicability of ICD-codes as a surveillance mission is restricted by disagreement and overlap among codes. For example, there is currently no ICD-code for bronchitis caused by IV infection, while "IV pneumonia" (J10.1, J11.1) is listed [41]. Moreover, the work by Alchikh et al. indicated that the surveillance based solely on ICD-codes risks overlooking the majority of ARI [36]. For example, more than three-fifths of IV cases and half of RSV cases are missing via ICD-codes, in addition to almost all cases of HRV, HAdV and HMPV infections [36]. It should also be noted that laboratory reports do not avoid observer bias, as laboratory reports depend upon a clinician's or practitioner's decision to collect specimens or order diagnostic tests [36]. Thus, specific psychological inclinations, which influence diagnostic reasoning, can result in mistakes in clinical practice [42]. Furthermore, it has been demonstrated that many diagnostic mistakes derive from faulty reasoning and not lack of professional knowledge [43]. Failures in cognitive reasoning account for nearly 30% of common mistakes involving interns

[44]. Thus, the physician's perceived feeling of whether diagnostic tests are needed may influence the patient's diagnosis. In addition, the lack of diagnostic test results does not mean there is no pathogen, because possible variations in the process of sampling and different quality of tests regarding low sensitivity may result in false negative results.

Alternatively, clinical case definitions based on clinical signs and symptoms are applied to perform on certain diagnostic tests among facilities [36, 45, 46]. Several international case definitions of ILI have been recommended by the Centers for Disease Control and Prevention (CDC) [47], ECDC [48] and the WHO [49], as well as an ARI case definition recommended by the ECDC [48] (**Table 1**). Poor and varying specificity (6.6%–21.4%) or sensitivity (36%–61%) of diagnosing IV using all four international case definitions have been previously evaluated by

Table 1: Different case definitions for ARI or ILI according to the CDC [47], ECDC [48], WHO[49, 51] and this study [36, 52, 53]

Name	Definition
CDC_ILI	Fever ≥38°C plus at least one of the following: cough and sore throat, and absence of a known cause other than influenza [47]
WHO_ILI (old)	Sudden onset of fever >38°C plus either cough or sore throat [51]
WHO_ILI (new)	An acute respiratory illness with a measured temperature of ≥38°C plus cough with onset within the last ten days [49]
ECDC_ILI	Sudden onset of symptoms plus at least one systemic symptom (including fever, feverishness, headache, malaise, myalgia) plus at least one respiratory symptom (including cough, sore throat, shortness of breath) [48]
ECDC_ARI	Sudden onset of symptoms plus one or more of the following symptoms (including cough, sore throat, shortness of breath and coryza) plus a clinician's judgement that the illness is due to an infection [48]
QM_ILI (This study)	Fever with a body temperature ≥38°C and ≥1 respiratory symptom (including cough, rhinitis/coryza, red/sore throat, ear ache, dyspnea, tachypnea, labored breathing, wheezing) or a documented clinician diagnosis of ILI [36, 52, 53].

QM: quality management

different teams [50]. In addition, these case definitions are not ideally suited for children with ILI or ARI due to their low sensitivity (0%–68%) for predicting respiratory viral infections, definitions are crucial to daily clinical practice, but are often limited by similar clinical features caused by many respiratory viral pathogens. Thus, the development of novel diagnostic algorithms for respiratory viral infections is urgently needed to adjust for selection bias, missing data and limitations in routine hospital surveillance [36].

There are two factors to account for this phenomenon that only a small portion of patients has been routinely tested for respiratory viral pathogens in clinical practice. First, there are currently no therapy options available for most respiratory viruses, except for neuraminidase inhibitors (NAIs) and cap-dependent endonuclease inhibitors (Baloxavir marboxil, licensed in Japan) [57] for influenza, which are mainly palliative. Treatment with NAIs can alleviate disease severity, shorten illness duration, reduce complications and lessen overall health care expense if treatment is initiated early [58]. For personalized therapy, and the control and prevention of infection, appropriate diagnostics should be conducted in a timely manner (within the first hours of admission) prior to initiating antiviral treatment [58]. Alternatively, several types of NAIs, such as Oseltamivir and Zanamivir can be administered as a chemoprophylaxis against IV infection with an estimated efficacy of 68%-89% [59, 60]. In the United States, oral Oseltamivir prophylaxis is used in infants ≥ 3 months of age, as well as all high-risk children, whereas Zanamivir prophylaxis is administered only in children ≥ 5 years of age [60, 61]. However, its utility is restricted by concerns about potential adverse effects, widespread resistance and antiviral availability [59]. Note that individuals who have been administered chemoprophylaxis might still be at risk for IV infection and are likely to transmit IV even though clinical illness was prevented [60, 62]. In contrast to IV, there is no antiviral medication currently available for RSV, even though RSV is the main viral pathogen in patients with ARI, particularly in children. Secondly, Palivizumab prophylaxis for RSV infection is administered only in the first two years of life to children at the highest risk for severe disease [63, 64], and its use is limited by the high costs [65]. Vaccination is the most effective way to prevent infections. Currently, licensed vaccines are available only for IV and no other respiratory viruses. Several documented studies have indicated that immunization against IV in young children can reduce the disease burden and infection rates [66, 67]. However, vaccination coverage is a major factor affecting the public health benefit from vaccination. A low vaccination coverage rate has been reported in recent studies among children and adolescents in Germany (4%-23.6%) [68, 69] and other European countries including Italy (5.1%) and France (13.8%) [70]. In addition, a vaccine

might become available in the next 5 to 10 years for RSV [71, 72]. Considering the limited options of specific antivirals as well as concerns regarding antiviral resistance, antiviral therapy and high medication costs must be targeted at patients with a specific infection, as they will benefit the most from antiviral therapy [73]. Therefore, accurate and timely diagnosing of IV infection (including differentiating IV infection from other types of respiratory viral infections) remains crucial to providing targeted antivirals.

Another major reason for reduced diagnostic testing is that diagnostic tests are expensive, not widely available, poorly sensitive, and too time-consuming. In clinical practice, three factors influence the evaluation of diagnostic tests: (1) test performance and quality (e.g., sensitivity and specificity), (2) laboratory feasibility including processing time, and (3) cost versus benefit [74, 75]. Currently, diagnostic techniques available for testing specific types of ARI or ILI include serological tests, virus culture, antigen detection tests (including rapid point-of-care assays) and molecular diagnostic tests including reverse transcription polymerase chain reaction (RT-PCR) and rapid molecular assays. Serological tests are not generally recommended since they do not provide timely results, and as such, do not facilitate clinical decision-making and are available only at a limited number of public health or research laboratories. Viral culture has historically been accepted as the "gold standard" for diagnosing a specific of ARI or ILI pathogen, such as IV, but it is limited by the availability of primary health care centers and also does not provide rapid results for clinical management. Molecular diagnostic methods, such as RT-PCR and rapid molecular tests, provide faster test results than virus cultures. Furthermore, RT-PCR ensures more accurate testing compared to rapid point-of-care tests. However, RT-PCR and other molecular assays are usually costly. It is worth noting that RT-PCR assays are not widely available outside of large academic and public health healthcare centers. Respiratory specimens need to be sent to specialized laboratories for RT-PCR, and there is a longer turnaround time for the test results. Moreover, even though the assay yields results in a few hours, the actual time may be considerably longer before the clinician receives the results.

In view of this, clinicians must utilize costly or poorly sensitive diagnostic tests to accurately diagnose IV and other respiratory viral infections in order to initiate early antiviral treatment if effective antivirals, such as NAIs or cap-dependent endonuclease inhibitors for influenza, are available. Alternatively, clinicians may follow CDC or ECDC guidelines on empirical antiviral treatment among those who are at high risk for a specific viral infection. Thus, novel

clinical decision algorithms to identify the risk in decision-making, including testing and treatment for IV and other respiratory viral infections, are urgently needed [76].

6.3 A novel diagnosis algorithm: the decision tree model

Data mining and machine learning have been widely accepted as effective and practical statistical methodologies to use to identify important associations between data obtained from various perspectives. Decision tree analysis is a crucial type of data mining and machine learning. It can create a model or algorithm that predicts the value of a target variable relative to several input variables [77]. Moreover, it can also be used to make classifications [77]. Decision tree analysis has the ability to not only interpret the interaction of these independent variables, has on influencing the outcome(s) of interest, but it can also facilitate various risk stratifications [78]. Moreover, the output of a decision tree algorithm provides visually intuitive information with respect to the hierarchical significance of the contributing variables being displayed from the top to the bottom of the tree (**Figure 1**) [79].

Most IV models used CART algorithms to predict the mortality of patients infected with highly pathogenic avian IV A (H5N1) [82], and the likelihood, as well as risk-stratification, of IV infection in patients with ARI [76, 79, 83, 84]. Among adults with suspected IV or ARI, a CART model was developed in which the presence of fever ≥ 37.3°C with chills/sweating predicted a high risk of IV infection; absence of fever ≥ 37.3°C alone predicted a moderate risk of IV infection; and absence of both (fever ≥ 37.3°C and chills/sweating) was more likely to predict a low-risk of IV infection [83]. This CART model produced an area under the receiver operating characteristic curve (AUC) value of 0.75–0.76 [83]. The CART algorithm, which included fever, cough and fatigue, yielded a sensitivity of 84% and specificity of 48% for predicting IV infections among individuals ≥ 5 years old with ARI [79]. In a retrospective study performed on 818 children with ILI between November 2012 and April 2013, symptoms, including cough, myalgia and diarrhea, and other variables, such as IV contact, IV incidence, and immunized status, were included in CART models to conduct the risk stratification of IV infection, which yielded a sensitivity of 84%–89% and specificity of 33%–46% [84]. However, low specificities (33%–48%) were observed in these studies using the CART algorithm [79, 84]. Prevalence data of respiratory viruses in the community combined with signs and symptoms have been suggested to be used for risk stratification and decisions regarding testing and treatment [83, 85].

In addition, underlying conditions are significant factors in treatment decisions for young patients who manifested with ILI [61]. Previous IV predictive models using CART algorithms utilized limited or minimal information of prevalence data regarding respiratory viruses and underlying conditions.

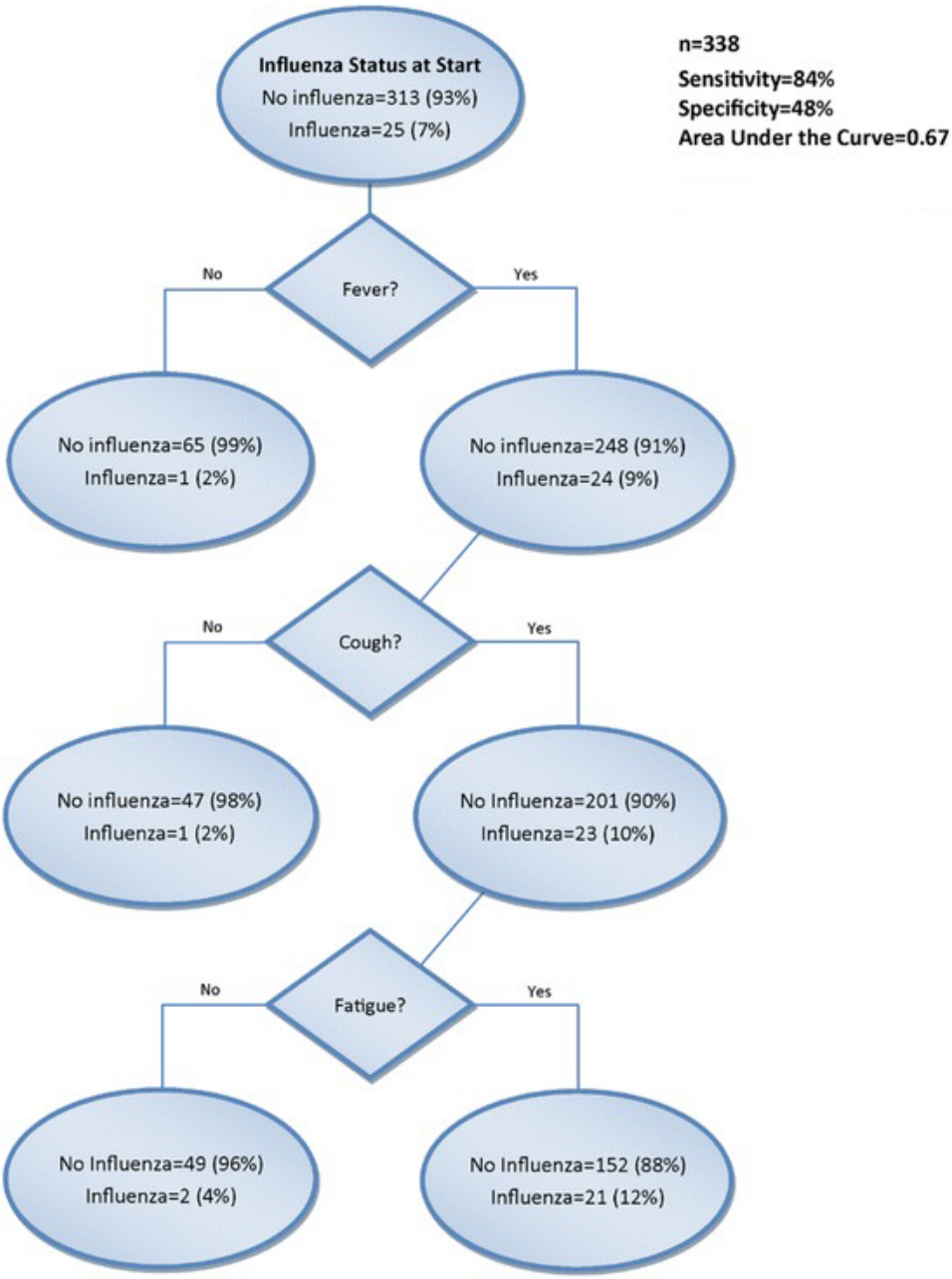


Figure 1: An example of classification and regression tree model for predicting IV infection [79]

Classification and regression tree (CART) [80] and conditional inference decision tree (CIDT) [81] are the two most important types of decision tree algorithms. Currently, CART algorithm has been frequently applied to predict IV but *not* other types of respiratory viral infections.

Most algorithms of decision tree recursive partitioning are special two-step cases, where the observational variables are initially partitioned via univariate splits in a recursive way, which is followed by fitting a constant model in each cell of the resulting partition [81]. CART and CIDT are the most commonly used algorithms. However, there are two fundamental disadvantages of the CART algorithm including overfitting and a selection bias towards covariates with numerous potentially possible splits [81, 86]. CIDT has successfully resolved these two problems [81]. Compared to the standard recursive partition algorithm used in CART, the CIDT algorithm is better used for the purpose of diagnosis [87]. As one type of decision tree algorithm, CIDT shares common functions including interpreting the interaction between multiple independent variables and risk-stratification. However, the CIDT has been rarely used for diagnosis and management in patients with ILI, especially for pediatric patients with ILI.

6.4 Objectives

Prompt diagnosis of IV and other respiratory viruses remains critical for patient management including effective antiviral therapy in patients with, or increased risk of, complications. However, highly sensitive and costly diagnostic tests, which are not widely available, and clinical decisions based on non-specific signs and symptoms make the diagnosis of IV and/or other respiratory viruses very challenging.

Currently existing international case definitions for ILI or ARI are not sensitive enough in children, thus novel diagnostic algorithms, such as the CIDT algorithm, are needed in order to better validate and improve case definitions of diagnosing IV and/or other respiratory viruses are urgently needed. Additionally, previous decision models for predicting ARI were limited solely to IV using the CART algorithm and did not develop predictive models for other types of ARI or consider the prevalence data and risk factors, such as underlying medical conditions, in the decision tree models.

Thus, this study's aims are as follows to:

- To investigate laboratory diagnoses, incidences and seasonal circulation of eight respiratory viruses, namely IV, RSV, HMPV, HRV, HAdV, HCoV, HPIV and HBoV-1, among children aged 0 to19 years in an inception cohort from December 2009 to April 2015.
- To identify key clinical features of the eight common respiratory viruses within a prospective inception cohort.
- To perform a literature review in order to examine the same question: key clinical features of the eight common respiratory viruses.
- To develop clinical decision rules in order to predict the risk of infection with one of the eight common respiratory viruses using a novel CIDT algorithm based on clinical features, (stepwise) plus risk factors, and (stepwise) plus seasonal circulation.

7 Materials and Methods

7.1 Materials

7.1.1 Technical equipment and consumables

The technical equipment and consumables (disposable materials, chemicals, enzyme and kits) used in this study are listed as follows (**Tables 2** and **3**).

Table 2: Technical equipment used in this study

Type of Equipment	Model	Source
Biosafety cabinet	Safe 2020 Class II	Thermoscientific, Hennigsdorf, Germany
Centrifuge	Heraeus Fresco 21 (refrigerated centrifuge)	Thermoscientific, Hennigsdorf, Germany
	Centrifuge 5424/ MiniSpin® plus (Mini centrifuge)	Neolab-Behr Labor-Technik, Heidelberg, Germany
	PerfectSpin P (Plate centrifuge)	VWR, Darmstadt, Germany
Freezer	4-8℃	Bosch, Denham, United Kingdom
	-20℃, ProfiLine	LiebHerr, Ochsenhausen, Germany
	-80℃, Forma 88000 series	Thermoscientific, Hennigsdorf, Germany
Heating block	BioShake iQ thermal mixer (life science unlimited)	Quantifoil Instruments GmbH, Jena, Germany
LightCycler	480 Instrument II 25032	Roche Diagnostics Deutschland GmbH, Mannheim, Germany
Pipettes	10µl, 20µl, 100µl, 200µl, 1000µl	EppendorfResearch® plus, Wesseling-Berzdorf, Germany
PCR workbench	In-house production	Robert Koch Institute, Berlin, Germany
Thermocycler	Biometra® T3000	Biometra GmbH, Göttingen, Germany
Vortexer	TEX-Genie® 2, 120V, (G560E)	Scientific Industries Inc, Karlsruhe, Germany

Table 3: Consumables

Type of method	Type of Consumables	Source
Nucleic acid extraction	Ethanol (96%)	Merck-VWR International GmbH, Dresden, Germany
	Invitek RTP® DNA/RNA virus Mini Kit (250)	Stratec Molecular GmbH, Birkenfeld, Germany
cDNA synthesis	dNTP/dNUTPs	Invitrogen Fisher Scientific GmbH, Schwerte, Germany
	Dithiothreolin (DTT)	Sigma-Aldrich, Munich, Germany
	Random primers	Metabion International AG, Planegg/Steinkirchen, Germany
	Reverse transcriptase	Invitrogen GmbH, Karlsruhe, Germany
	RNasin® RNase inhibitor	Promega, Mannheim, Germany
	5X RT Buffer	Invitrogen GmbH, Karlsruhe, Germany
Real-time RT-PCR	LighCycler® 480 Multiwell plate 96	Roche Diagnostics Deutschland GmbH, Mannheim, Germany
	Magnesium chloride ($MgCl_2$)	Invitrogen GmbH, Karlsruhe, Germany
	Platinum Taq DNA Polymerase	Invitrogen GmbH, Karlsruhe, Germany
	Sealing film (white)	Roche Diagnostics Deutschland GmbH, Mannheim, Germany
	10X PCR Buffer (2M Tris-HCl + 5M KCl)	Invitrogen GmbH, Karlsruhe, Germany
	BSA	PAA laboratories GmbH-VWR International GmbH, Dresden, Germany
Others	H_2O RNase free	Sigma-Aldrich, Munich, Germany
	Reaction tubes (0.5ml, 1.0ml and 1.5ml)	Eppendorf, Hamburg, Germany
	Tips for pipettes	Eppendorf, Hamburg, Germany

RT-PCR: reverse transcription PCR, dNTP: deoxynucleoside triphosphate, dUTP: deoxyuridine triphosphate.

7.2 Methods

7.2.1 Cohort dataset

7.2.1.1 Patients enrollment and sampling

Children aged 0 to19 with ILI were consecutively recruited into a prospective hospital-based inception cohort study during December 2009 to April 2015. The project was initiated by Barbara Rath, Vienna Vaccine Safety Initiative [52, 88-91]. The children candidates were enrolled from among outpatients of emergency room (ER) and inpatients of the department of paediatrics at Charité Medical University Berlin, Germany. A specifically trained quality management (QM) team screened all outpatients admitted to ER every Wednesday when private practice is usually closed in Germany [36]. All inpatients (including those from the intensive care unit and surgical department) were screened on a daily basis throughout the year by the same QM team. The study was approved by the Charité institutional review board (EA 4/008/10 and EA 24/008/10). Informed consent procedures were waived for the purpose of enhanced quality of care and infection control.

Pediatric patients who met the pre-defined criteria were enrolled into this study with voluntary participation. The criteria for ILI cases recruitment were defined as follows [36, 52, 53]: QM_ILI case definition was evidence of fever with a body temperature equal to or greater than 38℃ and at least one respiratory symptom (including cough, rhinitis/coryza, red/sore throat, ear ache, dyspnea, tachypnea, dyspnea, labored breathing and wheezing) or a documented clinician diagnosis of ILI (pediatric candidates could also be enrolled into the QM program if the treating clinician suspected ILI and requested enrollment in the QM program, regardless of the QM_ILI case definition).

The independent, specifically trained QM-personnel performed highly standardized clinical assessments for all enrolled patients using a disease severity score based on WHO criteria for uncomplicated and complicated/progressive disease [52, 92]. Complete physical examinations was executed: nasopharyngeal exam, oropharyngeal exam, auscultation of the lungs, otoscopy, and medical history including the status of vaccination [52]. Several clinical variables of importance were also well defined (**Table 4**).

Nasopharyngeal swab of enrolled patients were collected in universal transport medium (Copan TM, Copan Diagnostics, Murrieta, CA) by the special QM team and investigated at

the National Influenza Centre (NIC) at the Robert Koch Institute (RKI) for common respiratory viruses [52, 53, 88-92].

Table 4: Definitions for several symptoms used in this study [92]

Name of variables	Definition
Altered or loss of consciousness	Evidence of at least one of the following: (1) Central nervous system involvement (e.g. encephalopathy, encephalitis); (2) Altered mental status or unconsciousness (other than postictal) or dizziness or confusion; (3) Glasgow coma scale or infant face scale less than 15 and/or marked personality change; (4) Paralysis or severe weakness (including floppiness in infants); (5) Drowsiness or difficult to arouse (including lethargy and/or markedly reduced levels of activity).
Cough	Evidence of cough
Dehydration	Evidence of at least one of the following: (1) Severe dehydration (documented dehydration, need for IV-therapy or base excess less than -7 on blood gas analysis); (2) Decreased urine output and/or need for hemofiltration/ dialysis
Diarrhea	Diarrhea equal to or greater than 3 bowel movements or equal to or greater than 3 more per day or baseline
Dyspnea	Evidence of at least one of the following: (1) Shortness of breath (dyspnea, labored breathing, respiratory distress); (2) Difficulty breathing; (3) Tachypnea using age-appropriate standards (4) Mechanical ventilation or ECMO
Exacerbation of chronic disease	Exacerbation of chronic disease (including asthma, chronic hepatic, cardiovascular or renal disease, diabetes or other metabolic disease)
Fever	Any measurement in current disease episode equal to or greater than 38°C
Headache	Headache or pain in head/neck area on exam using age-appropriate techniques
Hemoptysis	Bloody/ colored sputum
High and prolonged fever	Body temperature greater than 40℃ for 3 days or more

Hypoxia	Evidence of at least one of the following: (1) Cyanosis (including turning blue during seizure); (2) Blood oxygen saturation less than 93%; (3) Oxygen-requirement (including blow-by oxygen); (4) Respiratory failure and/or need for medical ventilation or ECMO
LRTI/ superinfection	Evidence of at least one of the following: (1) LRTI (pneumonia, bronchitis, pulmonary rales, wheezing/obstruction, need for mechanical ventilation/ECMO including clinical, radiological); (2) Bacterial superinfection in the lower respiratory tract (laboratory, radiological, or clinical findings)
Malaise	Level of reduction in general well-being equal to or greater than 5 on a scale ranging from 0 to 10
Myalgia	Muscle pain on exam (including age- appropriate techniques in infants and children)
Need for hospitalization	Assessor's judgement that the patient should be admitted to an inpatient ward (regardless of cost, availability of hospital beds and other outside factors)
Need for ICU admission	Evidence of at least one evidence of the following: (1) Assessor's judgement that the patient would benefit from admission to the ICU (including intermediate care); (2) Assessor's judgement that the patient would benefit from assisted respiration (including BiPAP, CPAP), (3) Assessor's judgment that the patient would benefit from mechanical ventilation or ECMO
Pharyngitis	Sore throat or inflamed throat on exam
Rhinitis	Coryza or rhinitis on exam
Seizure	Evidence of seizures
Septic shock or multi-organ failure	Evidence of at least one of the following: (1) Septic shock; (2) Secondary complications (renal/multi-organ failure, rhabdomyolysis, myocarditis); (3) Hypotension and/or need for vasopressor support
URTI/ superinfection	Evidence of at least one of the following: (1) URTI (cough, coryza, red/sore throat, ear ache); (2) Bacterial infection in the upper respiratory tract (including laboratory, radiological, or clinical findings, such as purulent drainage, bulging tympanic membrane, positive Strep A rapid test or microbiology result)
Vomiting	Evidence of vomiting (at least once)

BiPAP: biphasic positive airway pressure, CPAP: continuous positive airway pressure, ECMO: extracorporeal membrane oxygenation, ICU: intensive care unit, LRTI: lower respiratory tract infection, URTI: upper respiratory tract infection.

7.2.2 Detection of respiratory viruses

7.2.2.1 Extraction of nucleic acid

Nucleic acid was extracted using RTP DNA/RNA Virus Mini Kit (Invitek, Germany) in accordance with the manufacturer's instructions using a specimen volume of 400 µl [53]. The total elution volume was 60µl. For reasons of internal-control, each sample of extraction was spiked by 20 µl of Feline Calicivirus [93].

7.2.2.2 Reverse transcription of viral RNA (cDNA synthesis)

Twelve-point five microliters of extracted RNA was subjected to complementary DNA (cDNA) synthesis by reverse transcription in a total reaction volume of 20 µl containing 200U Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invirogen, USA), 200U RNasin, 500nM random hexamer primers, 0.2mM deoxynucleoside triphosphate (dNTP), 2.5mM dithiothreitol and 1X Buffer (containing 250mM Tris-HCl [PH 8.3], 37.5mM KCl and 15mM $MgCl_2$). The reaction was performed on a Biometra T3000 themocycler for 5 minutes at 42°C, followed by 30 minutes at 37°C and finally 5 minutes at 95°C. The yielded cDNA (20 µl) was diluted with RNase-free H_2O (20 µl), producing a total of final 40µl diluted cDNA. Real-time RT-PCR amplification was used to detect respiratory viruses.

All specimens were analyzed for viral RNA of IV type A (H3N2 and H1N1 pdm 2009) and B (Yamagata and Victoria), RSV, HMPV, HAdV, HRV, HBoV-1, HCoV and HPIV by real-time RT-PCR assays. In-house multiplex real-time RT-PCR assays were used for testing for IV types A and B, RSV types A and B, HMPV genetic lineages A and B and HAdV species A to F; but positive results were not further distinguished for this study. Testing for influenza A and B viruses, RSV, HMPV, HAdV and HRV was performed by the NIC, RKI and primarily by Eleni Adamou, Charité Medical University as published previously [36, 92]. In this study, all specimens were investigated for HBoV-1, HCoV and HPIV by Xiaolin Ma (**Table 5**). These assays were designed by Barbara Biere, RKI, Germany.

Testing for HCoV (NL63, 229E, OC43 and HKU1), HPIV1-4 and HBoV-1 was performed in a total reaction of 15µl containing 1x PCR buffer, 4mM magnesium chloride ($MgCl_2$), 0.2mM dNTP with dUTP, 40ng/µl bovine serum albumin (BSA), 0.3U platinum Taq polymerase, primers and probes (**Table 5**), and 5 µl of cDNA (or nucleic acid for HBoV-1) [53]. Amplification was carried out at 95 °C for 300 seconds, followed by 45 cycles at 95 °C for 15 seconds and 60 °C for 30 seconds [53].

Table 5: Oligonucleotide sequences for the detection of HCoV, HPIV and HBoV-1[53]

Virus	Primer	sequence (5´→3´)	nM
HCoV-NL63	Forward	AACgTgTTgATTTgCCTCCTAA	300
	Reverse	gTTTgCgATTACCAAgACTgg	300
	TMGB	CTTATgAggTCCAgTACC	100
HCoV-229E	Forward	TACCACACTTCAATCAAAAgCTCC	300
	Reverse	gCgACTCTgMgACCTYgACT	300
	TMGB	CACgggAgTCAggTTCT	100
HCoV-OC43	Forward	CGATGAGGCTATTCCGACTAGGT	300
	Reverse	CCTTCCTGAGCCTTCAATATAGTAACC	300
	TM	TCCGCCTGGCACGGTACTCCCT	100
HCoV-HKU1	Forward	CTTGCGAATGAATGTGCWCAAG	300
	Reverse	TTGCATCACCACTGCTAGTACCAC	300
	TM	TGTGTGGCGGTTGCTATTATGTTAAGCCTG	100
HPIV-1	Forward	TGCAATATATGCRTATTCATCAAACTTAAT	300
	Reverse	CTAATTGTAAAACCTGATATGACTTCCCTA	300
	MGB	ACTCAAGGATGTGCAGATA	100
HPIV-2	Forward	ATCTTCAGGACTATGAAAACCATTTACC	300
	Reverse	CACAACCTCCTGGTATAGCAGTGAC	300
	TM	AAGTGATGGAATCAATCGCAAAAGCTGTT	100
HPIV-3	Forward	GCATTGTATCATCTGTCATATTRGAYTCAC	300
	Reverse	GCCAGCTCGTTYACYCTTTCRGT	300
	TM	TCGAGAGTBAACCCAGTCATAACTTACTCAACA	100
HPIV-4	Forward	AGACGTCTCAAAATTTGTTGATCAAG	300
	Reverse	GGTTCCAGAYAAWATGGGTCTTGCTA	300
	MGB	TCAAGTGTAATTGTATTRTC	100
HBoV-1	Forward	TACAAAAGAAAAGGGAGTCCAGAAA	300
	Reverse	TCCTGCTCCTGTGATGAGTTGT	300
	MGB	CCAgTgTCTCTTCCT	100

Oligonucleotides of primers and TaqMan probes were provided by Metabion International AG, Planegg/Steinkirchen, Germany). TMGB probes were provided by Applied Biosystems (Foster City, US).

7.2.3 Literature review dataset

This part has been published in the journal of **Reviews in Medical Virology** [53].

7.2.3.1 Literature search and selection criteria

We searched the English language literature published in Medline (Pubmed) from January 01, 1996 to Mar 21, 2017 [53]. The following search terms were used: "(((Influenza A[Title/Abstract] OR Flu A H3N2[Title/Abstract] OR Flu A H1N1[Title/Abstract] OR Flu A H1N1 2009 pdm[Title/Abstract] OR influenza B[Title/Abstract] OR Flu B Yamagata[Title/Abstract] OR Flu B Victoria[Title/Abstract] OR rhinovirus[Title/Abstract] OR HRV[Title/Abstract] OR RV[Title/Abstract] OR adenovirus[Title/Abstract] OR ADV[Title/Abstract] OR human metapneumovirus[Title/Abstract] OR hMPV[Title/Abstract] OR respiratory syncytial virus[Title/Abstract] OR RSV[Title/Abstract] OR human coronavirus[Title/Abstract] OR human coronavirus NL63[Title/Abstract] OR human coronavirus 229E[Title/Abstract] OR human coronavirus OC43[Title/Abstract] OR human coronavirus HKU1[Title/Abstract] OR HCoV[Title/Abstract] OR HCoV-NL63[Title/Abstract] OR HCoV-229E[Title/Abstract] OR HCoV-OC43[Title/Abstract] OR HCoV-HKU1[Title/Abstract] OR huamn bocavirus 1[Title/Abstract] OR HBoV-1[Title/Abstract] OR parainfluenza virus 1-4[Title/Abstract] OR PIV[Title/Abstract] OR PIV 1[Title/Abstract] OR PIV 2[Title/Abstract] OR PIV 3[Title/Abstract] OR PIV 4[Title/Abstract] OR respiratory virus[Title/Abstract])) AND (clinical features[Title/Abstract] OR clinical manifestations[Title/Abstract] OR clinical characteristics[Title/Abstract] OR clinical characterization[Title/Abstract] OR clinical outcomes[Title/Abstract] OR clinical presentations[Title/Abstract] OR symptoms[Title/Abstract])) AND (neonate[Title/Abstract] OR newborn[Title/Abstract] OR infant[Title/Abstract] OR child[Title/Abstract] OR children[Title/Abstract] OR adolescent[Title/Abstract] OR teenager[Title/Abstract] OR toddlers[Title/Abstract] OR age < 18 years old[Title/Abstract])" [53]. The literature search was conducted on 21/03/2017, and the strategy for search was registered at the International Prospective Register of Systematic Reviews (No. CRD42017059557) [53].

The publications identified by the initial search were reviewed by Xiaolin Ma, and the studies included had to meet the following criteria [53]: providing data on children aged 0 to 18 years; clinical trials (randomized/non-randomized), observational studies or epidemiological reports;

and one or more reporting association(s) between confirmed positive as well as negative viral infection and a clinical feature.

Publications were excluded if any of the following criteria were met[53]: animal studies, in vitro studies, adult studies or case series; lacking reporting data regarding symptoms/signs and outcomes, lacking reporting virological data; lacking reporting data of corresponding virus-negative control groups, reporting data not available to be categorized and extracted; overlapping studies addressing chronic conditions or other non-respiratory infections; and meta-analyses, review papers, and conference papers.

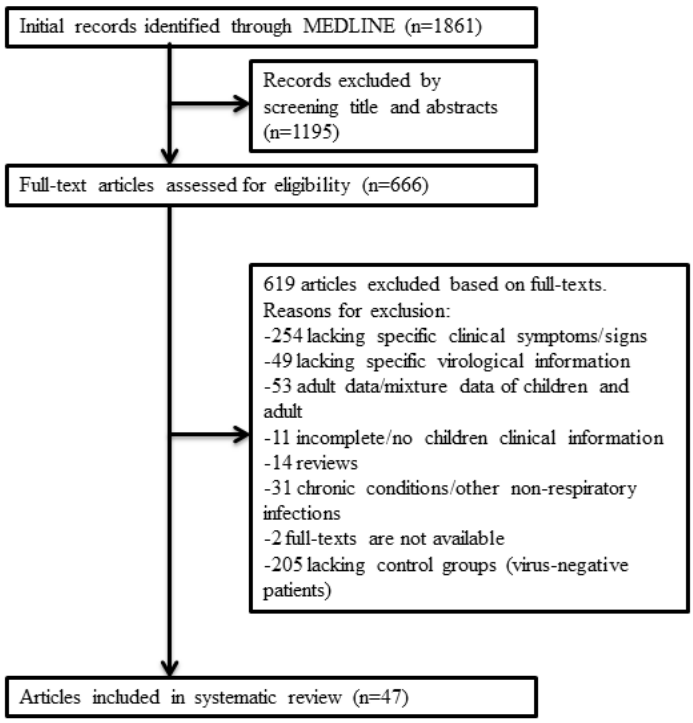


Figure 2: Flow chart of a selection of publications in the systematic literature dataset [53]

Searches identified 1861 publications from Pubmed. After initially filtering and deleting the duplicates on the basis of titles and abstracts, a total of 666 relevant publications remained. After reviewing, additional 619 publications were eliminated according to the pre-defined inclusion and exclusion criteria. Consequently, a total of 47 relevant publications were included for the systematic literature review **(Figure 2)**.

7.2.3.2 Data extraction and management

Full-text versions of all relevant publications were obtained and assessed as follows. Data were extracted by Xiaolin Ma and checked by Barbara Rath. Any relevant disagreement was resolved by discussion and consensus among the reviewer team (Xiaolin Ma, Mareen Alchikh and Barbara Rath).

The data extracted included the following information [53]: (1) first author and year of publication, (2) country of study location, (3) study design, (4) age of patient population, (5) number of subjects included, (6) type of sampling and laboratory method, (7) clinical presentations: any presenting symptoms and sings (including respiratory and extra-respiratory signs and symptoms). The clinical presentations were classified into the following nineteen distinct groups [53]: altered or loss of consciousness (altered/LOC), anorexia/difficulty feeding (anorexia/DF), apnea, conjunctivitis, cough, hypoxia, diarrhea, dyspnea, fever, headache, malaise, myalgia, rash, rhinitis, seizures, sore throat, signs of URTI, vomiting and wheezing/ signs of LRTI.

7.2.4 Statistical analysis

The statistical analyses, including odds ratio (OR) analysis and CIDT algorithm, were conducted by Tim Conrad (Free University, Berlin, Germany) based on R with the metaphor Package software [94]. SPSS 16.0 was used to analyze the variations of annual- and age-related incidence for all eight respiratory viruses. The analyses were conducted by Janine Reiche, RKI, Berlin, Germany. *P*-values less than 0.05 were considered statistically significant.

In the literature dataset, OR meta-analysis was performed on pooled data regarding the relation between clinical features and viral pathogens [53]. Random effect models for meta-analysis were applied due to heterogeneity across all studies [53, 94]. Pooled results as well as individual study results were illustrated by forest plots. I^2 statistics was used to test heterogeneity across all studies (I^2 values < 25%, 25-75% and >75% were considered low, medium and high levels of heterogeneity, respectively) [53, 95]. Publication bias was assessed by using funnel plots (A symmetrical plot indicates a lack of publication bias) [53, 96]. In the cohort dataset, clinical features associated with viral pathogens were also conducted by using OR analysis [53].

In the cohort dataset, a CIDT algorithm for unbiased recursive partitioning was used to explore and evaluate the diagnostic capacity for specific viral infections [81]. Briefly, the CIDT algorithm identified the predictor variable that best differentiates between patients with and without the outcome of interest. The program employed the same analysis among the subgroups created in the previous step. The process continues until a stopping criterion exists, in which a pre-specified minimum group size is reached or the process is stopped by the investigator. In CIDT algorithm, stopping criteria in light of multiple test procedures were carried out and the predictive property of the resulting trees was as good as the property of established comprehensive search procedures [81]. The output after stopping criteria is a terminal node, which collectively generates a decision tree that shows the variables and interaction between them in predicting the outcome of interest. Each node has a probability of the outcome of interest. For each of the decision models, the overall predictive capacity (accuracy, sensitivity and specificity) to discriminate between patients and without specific viral infection was assessed using AUC. In this study, the collected data of clinical examination and laboratory virological detection comprised 40 dichotomous attributes for each specific infection of the eight common respiratory viruses, including (1) 22-item clinical variables: altered/LOC, cough, dehydration, diarrhea, dyspnea, exacerbation of chronic disease, fever, headache, hemoptysis, high and prolonged fever, hypoxia, LRTI/superinfection (henceforth labeled "LRTI"), malaise, myalgia, need for hospitalization, need for ICU admission, pharyngitis, rhinitis, seizure, septic shock or multi-organ failure, URTI/superinfection (henceforth labeled "URTI"), vomiting; (2) 15-item risk factors: children younger than 2 years of age, and 14-item underlying medical conditions (chronic pulmonary condition, chronic cardiac condition, chronic renal disease, chronic hepatic disease, chronic neurological condition, diabetes, obesity, other metabolic condition, hemoglobinopathies, congenital immunosuppression, acquired immunosuppression, aspirin therapy, pregnancy and prematurity < 33 weeks gestational age); and (3) three levels (low/moderate/high) of seasonal pattern. It is worth noting that these 15-item risk factors have been successfully assessed by Barbara Rath et al. [92] as a risk factor for eight respiratory viral infections in this inception cohort.

8 Results

8.1 Demographic features of patients

8.1.1 Composition of inception cohort

From December 2009 to April 2015, an estimated total of 139,000 patients under 19 years of age were referred to the Emergency Department of Pediatrics or inpatient ward in Charité Medical University in Berlin, Germany [36]. Out of these patients, 6073 (around 4.7%) met the ILI case definition criteria and were enrolled in the inception cohort (**Figure 3**). Of 31 patients, it was inappropriate to collect specimens. 6042 specimens were tested for one or more respiratory viruses to determine the cause of ILI (**Figure 3**).

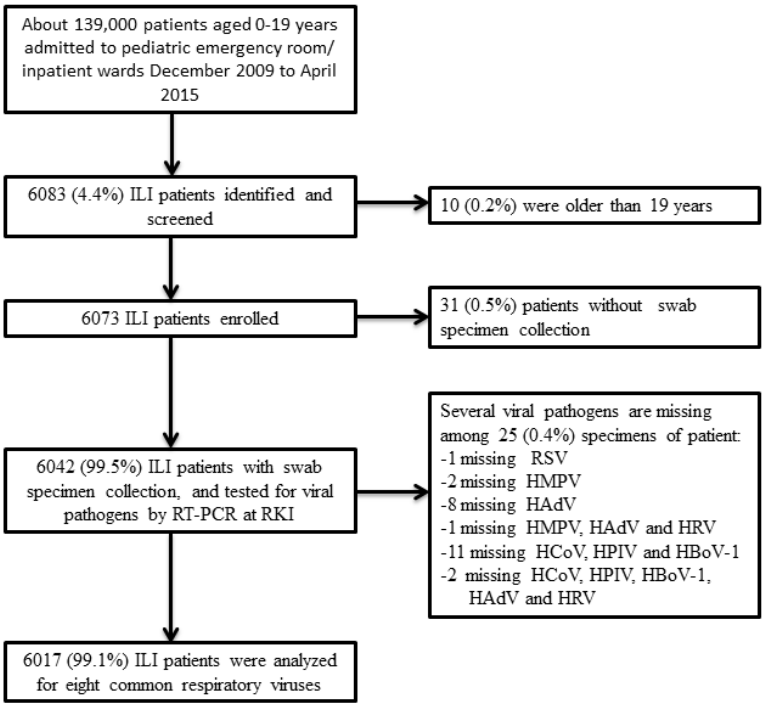


Figure 3: Flow chart of case enrollment among pediatric emergency room/inpatient wards enrolled in inception cohort in Charité Medical University

8.1.2 Demographic features of ILI patients

The median age of the enrolled ILI patients was 1.2 years (range 0.0-18.8 years), 57.3% of participants were under two years old, and 56% were male (**Table 6**) [92]. Of 6042 subjects, 25.8% had an underlying medical condition, the top 3 of which were chronic pulmonary condition (8.1%), cardiac condition (8.0%) and neurological condition (5.5%) (**Table 6**). In

addition, 1685 (27.8%) and 202 (3.3%) patients received antibiotic and antiviral treatment, respectively; and 8.2% were vaccinated against influenza [36, 92].

Table 6: Demographic features and underlying medical conditions of 6042 children with ILI

Features	Number (%)
Male	3382 (56.0%)
Median age, years (range)	1.2 (0.0-18.8)
Age group	
<2 years	3466 (57.4%)
2-4 years	1445 (23.9%)
5-19 years	1131 (18.7%)
At least one underlying medical condition	1558 (25.8%)
Acquired immunosuppression condition	46 (0.8%)
Aspirin therapy	58 (1.0%)
Cardiac condition	481 (8.0%)
Chronic renal disease	151 (2.5%)
Chronic hepatic disease	47 (0.8%)
Chronic neurological condition	335 (5.5%)
Congenital immunosuppression condition	99 (1.6%)
Diabetes	18 (0.3%)
Hemoglobinopathies	50 (0.8%)
Obesity	76 (1.3%)
Other metabolic condition	157 (2.6%)
Pulmonary condition	487 (8.1%)
Pregnancy	2 (0.03%)
Prematurity < 33 weeks gestation	316 (5.2%)

8.2 Clinical features of ILI patients

Fever≥38^{o}C (88.9%), cough (74.9%), rhinitis (70.8%), sore/inflamed throat (67.3%) and dyspnea (47.7%) were the most common symptoms of patients with ILI at admission. Of all ILI patients, 12.3% had high fever and prolonged fever at admission: body temperature >40^{o}**C** for three days or more. Of 6042 ILI patients, 4040 (66.5%) needed to be hospitalized, and 997 (16.4%) needed to be admitted to the ICU. URTI was more frequently reported than LRTI (91.5% versus 46.6%) (**Table 7**).

Table 7: Clinical features of 6042 pediatric patients with ILI at presentation

Clnical Features	Number (%)
Altered or loss of consciousness	497 (8.2%)
Anorexia or difficulty feeding	2581 (42.7%)
Apnea	265 (4.4%)
Conjunctivitis	530 (8.8%)
Cough	4526 (74.9%)
Dehydration	873 (14.4%)
Diarrhea	959 (15.9%)
Dyspnea	2880 (47.7%)
Exacerbation of chronic disease	186 (3.1%)
Fever (≥38^{o}C)	5370 (88.9%)
Fever (>40^{o}C) for 3 days and more	745 (12.3%)
Headache	654 (10.8%)
Hemoptysis	103 (1.7%)
Hypoxia	1510 (25.0%)
Lower respiratory tract infection/ superinfection	2816 (46.6%)
Malaise	2347 (38.8%)
Myalgia	209 (3.5%)
Need for hospitalization	4016 (66.5%)
Need for ICU admission	1310 (21.7%)
Pharyngitis (sore/inflamed throat)	4068 (67.3%)
Rash	840 (13.9%)
Rhinitis	4280 (70.8%)
Seizure	656 (10.9%)
Septic shock or multi-organ failure	38 (0.6%)
Vomiting	1879 (31.1%)
Upper respiratory tract infection/ superinfection	5531 (91.5%)

8.3 Detection of respiratory viruses

8.3.1 Incidence and age distribution

6042 specimens were investigated for IV (including influenza A and B viruses), RSV, HMPV, HAdV and HRV, HPIV (including HPIV 1-4), HCoV (including HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43) and HBoV-1 using in-house multiplex real-time RT-PCR.

On average, 93 specimens were tested each month, with peaks during the winter months (**Figure 4**). Of the 6042 specimens investigated, 4166 (70.0%) were positive for at least one

viral pathogen. Overall, HRV (22.4%) was most frequently detected, followed by RSV (17.1%), HBoV-1 (15.7%) and IV (11.1%) (**Table 8**). The least detected pathogen was HMPV (4.4%). Over seven consecutive seasons, incidences varied significantly by virus, except for HPIV. IV showed an intensive circulation in odd years and weak circulation in even years (p=0.001). Conversely, RSV and HCoV, an intensive circulation was determined in even years, and a weak circulation in odd years (with exception of the year 2015) (both p-values=0.001) (**Table 8**). Intensive circulation was observed every two years for HAdV (p=0.002). For HRV, HBoV-1 and HMPV, no regular pattern of intensive or weak seasonality was observed.

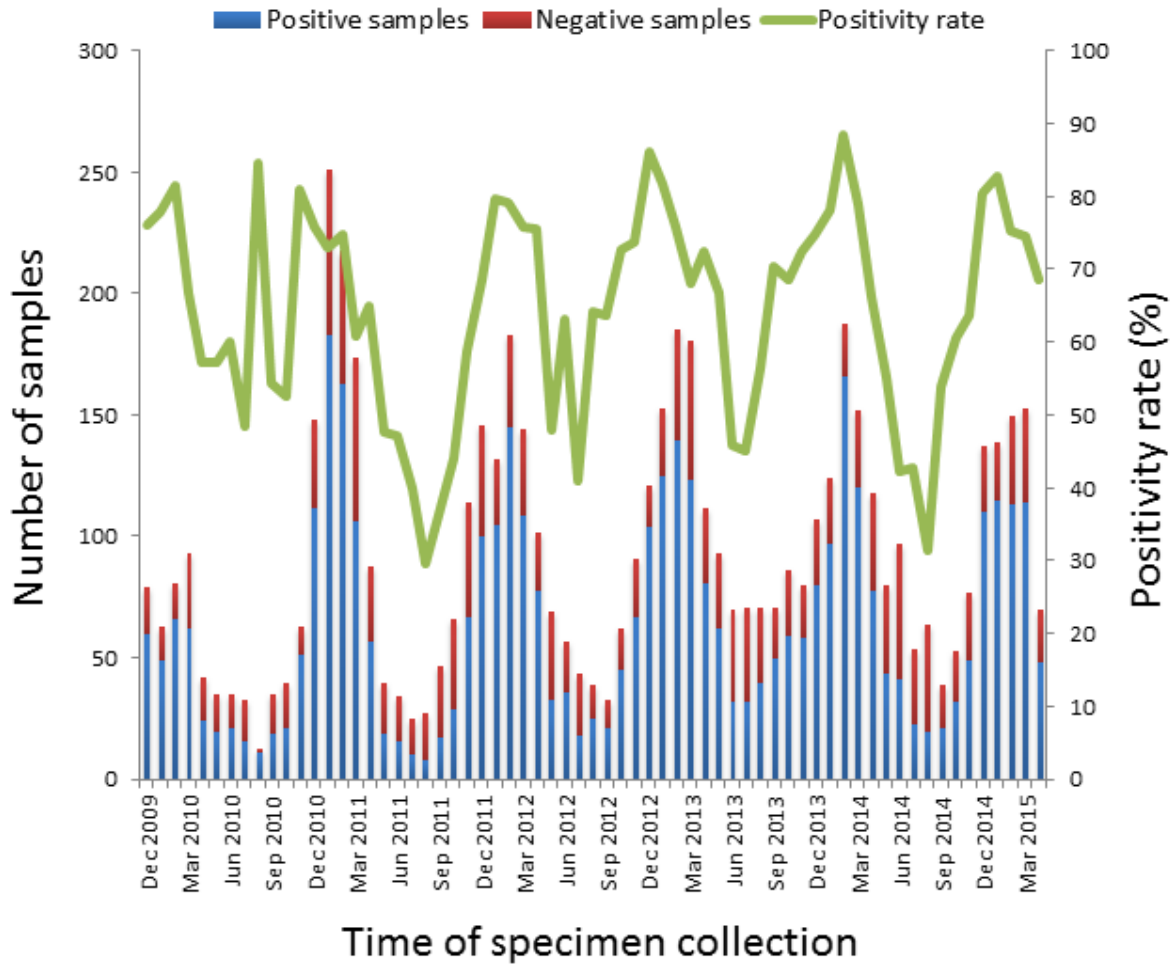


Figure 4: Number of specimen processed per month. Positive samples (blue bars) versus negative samples (red bars) and positivity rate (line graph) each month during December 2009 to April 2015. Positive samples are defined as those that showed at least one respiratory viral infection.

Table 8: Incidence of respiratory viruses by year

Year	NS	NPS (%)	Number (%) of viral pathogens detected							
			HRV	RSV	HBoV-1	IV	HAdV	HPIV	HCoV	HMPV
2009	79	60 (75.9)	17 (21.5)	8*** (10.1)	13 (16.5)	14*** (17.7)	13** (16.5)	4 (5.1)	0***	12** (15.4)
2010	681	472 (69.3)	156** (22.9)	138*** (20.3)	125*** (18.4)	24*** (3.5)	85** (12.5)	42 (6.2)	50*** (7.4)	31 (4.5)
2011	1230	775 (63.0)	226** (18.4)	139*** (11.3)	186*** (15.2)	203*** (16.5)	102** (8.3)	80 (6.5)	26*** (2.1)	43** (3.5)
2012	1077	786 (73.0)	254** (23.6)	237*** (22.0)	231*** (21.5)	84*** (7.8)	90** (8.4)	66 (6.1)	63*** (5.9)	23** (2.1)
2013	1280	882 (68.9)	308** (24.1)	133*** (10.4)	180*** (14.1)	190*** (14.8)	125** (9.8)	95 (7.5)	40*** (3.1)	54 (4.2)
2014	1183	801 (67.7)	306** (25.9)	268*** (22.7)	147*** (12.4)	49*** (4.1)	115** (9.8)	76 (6.4)	60*** (5.1)	68** (5.7)
2015	512	390 (76.2)	86** (16.8)	113*** (22.1)	62*** (12.1)	109*** (21.3)	40** (7.8)	15 (2.9)	36*** (7.0)	33** (6.4)
Total	6042	4166 (70.0)	1353 (22.4)	1036 (17.1)	944 (15.7)	673 (11.1)	570 (9.5)	378 (6.3)	275 (4.6)	264 (4.4)
p-value	-	-	0.006[a]	0.001[b]	0.001[b]	0.001[b]	0.002[b]	0.071[b]	0.001[b]	0.003[b]

NOS: number of specimen, NOPS: number of positive specimen.
[a] *p*-value was calculated using the Chi-squared test by Pearson method.
[b] *p*-value was calculated using the Chi-squared test by Monte-Carlo method.
*: statistically significant, $p < 0.05$; **: very statistically significant, $p \leq 0.01$; ***: highly statistically significant, $p \leq 0.001$.

The distribution of respiratory viral pathogens was analyzed with regard to the age of children. Three age categories were investigated: (1) children younger than 2 years, (2) children aged 2-4 years, and (3) children aged 5-19 years. In this inception cohort, children with ILI younger than 2 years of age were infected predominantly with RSV, HAdV, HRV, HBoV, HPIV and HCoV ($p \leq 0.001$), while children aged 2-4 years and equal to and older than 5 years were most likely to be infected with IV ($p = 0.001$) (**Table 9**).

Table 9: Incidence of respiratory viruses by age

Age	TONP	Number (%) of viral pathogens detected							
		HRV	RSV	HBoV-1	IV	HAdV	HPIV	HCoV	HMPV
<2ys	3466	848*** (24.2)	812*** (23.4)	632*** (18.2)	195*** (5.6)	374*** (10.8)	246*** (7.1)	197*** (5.7)	159 (4.6)
2-4ys	1445	350 (24.2)	196*** (13.6)	219 (15.2)	218*** (15.1)	149 (10.3)	97 (6.7)	59 (4.1)	78** (5.4)
≥5ys	1131	155*** (13.7)	28*** (2.5)	93*** (8.2)	260*** (23.0)	47*** (4.2)	35*** (3.1)	19*** (1.7)	27** (2.4)
p-value	-	0.001[a]	0.001[b]	0.001[b]	0.001[b]	0.001[b]	0.001[b]	0.001[b]	0.002[b]

TONP: total number of patient, ys: years.
[a] *p*-value was calculated using the Chi-squared test by Pearson method.
[b] *p*-value was calculated using the Chi-squared test by Monte-Carlo method.
*: statistically significant, $p < 0.05$; **: very statistically significant, $p \leq 0.01$; ***: highly statistically significant, $p \leq 0.001$.

8.3.2 Single infection and co-infection(s) with respiratory viruses

Mono- and co-infection with viruses were assessed (**Table 10**). Of 4156 positive specimens for which all eight viruses were investigated, 3020 (72.7%) single viral infections and 1134 (27.3%) multiple viral infections were identified. Co-infections included 967 (23.3%) double infections, 143 (3.4%) triple infections, 23 (0.6%) quadruple infections and 1 (0.02%) quintuple infection. HRV, RSV and IV were the top three of single pathogens identified. Co-infections were detected for all eight pathogens, among them HBoV-1, HRV and RSV were predominant (**Table 10**). The only one quintuple infection was the combination of HRV, HBoV-1, HAdV, HCoV and HPIV. The combinations of dual, triple and quadruple viral infections are shown in **Table 11**, **Figure 5a** and **Figure 5b**.

Table 10: Determinations of single and multiple virus detection in 4156 positive patients[§]

Virus	NOS (%)	HRV	RSV	HBoV-1	IV	HAdV	HPIV	HCoV	HMPV
Single	3020 (72.7)	**829**	**624**	**292**	**517**	**249**	**243**	**106**	**162**
Multiple	1134 (27.3)	521	410	652	154	320	133	169	101
Double	967 (23.3)	424	321	518	142	229	108	111	81
Triple	143 (3.4)	78	74	115	10	71	22	44	15
Quadruple	23 (0.6)	18	15	18	2	19	2	13	5
Quintuple	1 (0.02)	1	-	1	-	1	1	1	-
Total	4156 (100)	1350	1034	944	672	569	376	275	263

NOS: Number of specimen.
§: All eight viruses were investigated in 4156 specimens.

Table 11: Distributions of single and double virus detection in 4156 positive patients[§]

Virus	HRV	RSV	HBoV-1	IV	HAdV	HPIV	HCoV	HMPV
HRV	**829**	123	205	20	141	66	47	36
RSV	123	**624**	221	9	64	15	68	14
HBoV-1	205	221	**292**	104	132	45	65	34
IV	20	9	104	**517**	18	2	6	9
HAdV	141	64	132	18	**249**	22	36	19
HPIV	66	15	45	2	22	**243**	9	3
HCoV	47	68	65	6	36	9	**106**	11
HMPV	36	14	34	9	19	3	11	**162**

§: All eight viruses were investigated in 4156 specimens.

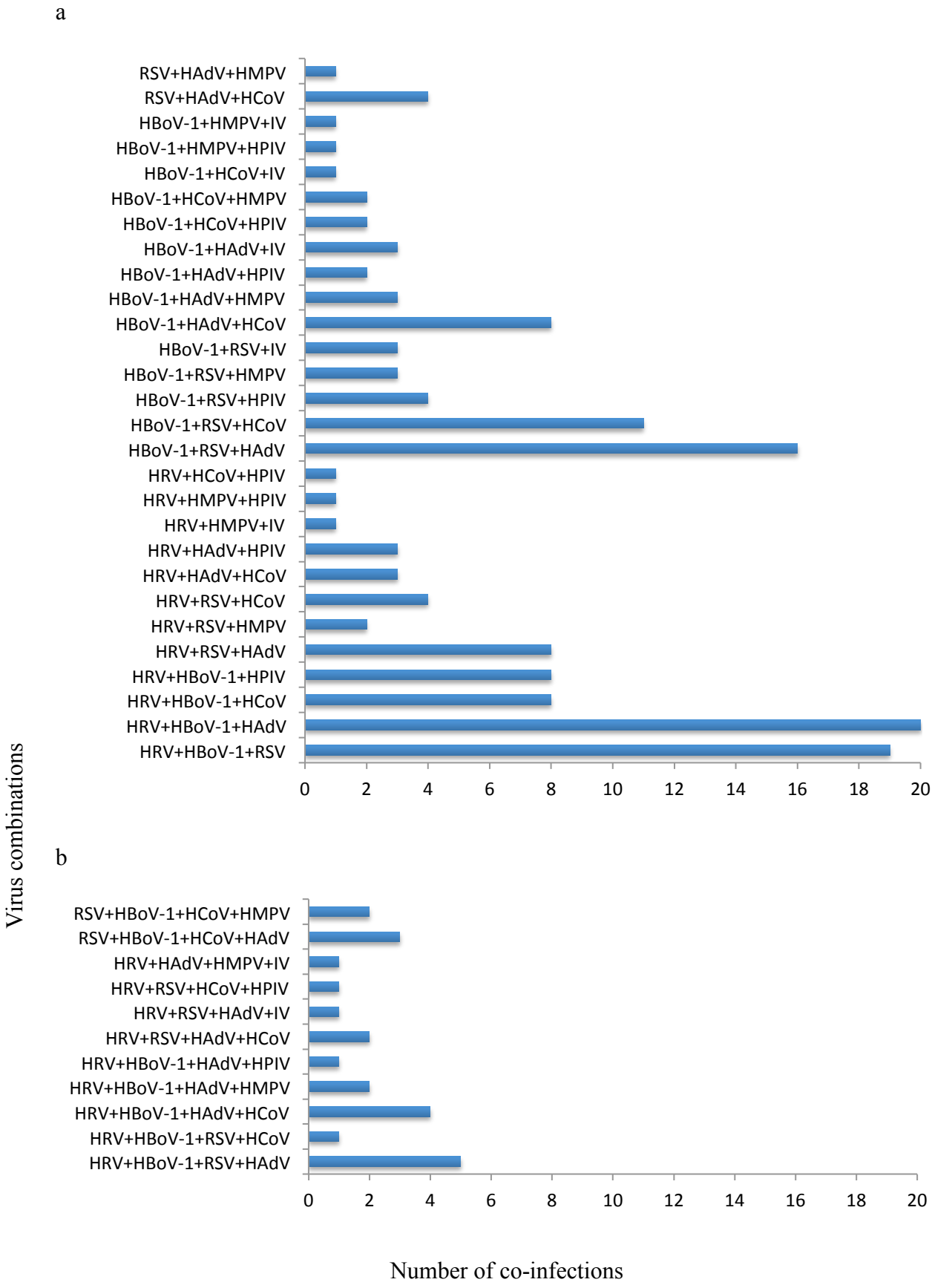


Figure 5: Virus combinations of (a) triple and (b) quadruple infections

8.4 Seasonal patterns of respiratory viruses

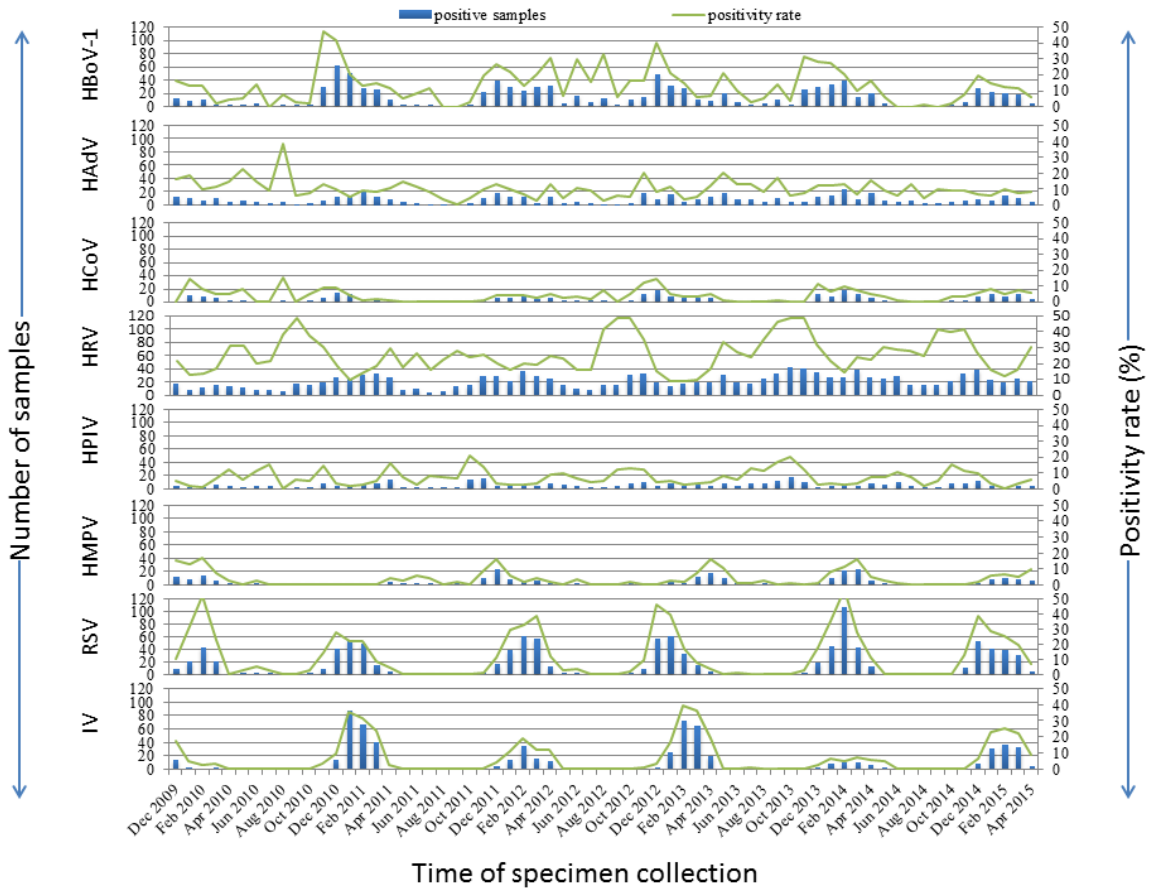


Figure 6: Detection of respiratory viruses in children with influenza-like illness from December 2009 to April 2015.

The seasonal patterns of respiratory viral infections were assessed by using the admission dates for the children (**Figure 6**). IV had a strong seasonal pattern between winter and early spring, with a peak in January or February. RSV also showed a strong seasonal activity and was more likely to be seen from late autumn to spring and peaked in December or February. A regular 2-year cyclic rhythm with occurrence of alternating early and late season was observed. Early season onset in October (2010/2011 and 2012/2013 season) was followed by late season onset in November (2011/2012 and 2013/2014 season). Significant association between onset and severity in the course of RSV epidemic season: Early onset for intensive season and late onset for weak season ($p \leq 0.001$, **Table 8**). An exception was the 2014/2015 season (an intensive epidemic RSV season), in which late RSV season onset (November) was observed. HMPV often followed a regular pattern within winter months and a disappearance during summer months. HPIV occurred from spring to autumn and often declined between

December and March. HRV occurred throughout the year and had a higher incidence in autumn months.

HAdV, HBoV and HCoV occured all the year round and showed no clear seasonality. It is worth noting that there was variation for HCoV: there was no occurrence between mid-spring and mid-autumn every two years.

8.5 Associations between viral infections and clinical features

Published studies have investigated the associations between certain respiratory pathogens and individual clinical features. In order to further identify which features are most commonly associated with a specific viral infection, a literature review was performed and clinical features were analyzed (**1.5.2**), and then compared with inception cohort dataset (**1.5.3**). In addition, these data have been published [53].

8.5.1 Characteristics of the literature review dataset

Characteristics of 47 finally included publications are summarized in **Table 12** [6, 97-142], yielding 9960 individual cases of laboratory-confirmed ARI and 39898 cases with negative test results for the same virus, respectively [53]. Twelve out of 47 studies (25.5%) recruited infants and children aged 0-5 years, 3 out of 47 (6.4%) recruited infants and children aged 0-3 years, 6 out of 47 studies (12.8%) recruited infants and children aged 0-2 years [53]. Laboratory detections of pathogen(s) varied within and between studies, including PCR (40/45, 88.9%), culture (3/47, 6.4%), specific reagent or enzyme-linked immunoassay (7/47, 14.9%) and direct/indirect immunofluorescence (10 /47, 21.3%) assay [53]. Of 47 studies, 16 studies (34.0%) were derived from the WHO Region of the Americas, followed by ten (21.3%) studies from the European Region and the WHO Western Pacific Region (21.3%), respectively. Four studies were reported in African Regions (8.5%) and Eastern Mediterranean Regions (8.5%), and three (6.4%) studies in the South-East Asia Region [53].

Table 12: Characteristics of the 47 enclosed publications [53]

Author, year, reference	Country	Design	Size	Age	Sample type	Type of RTI	Lab method	Pathogen
Ahn 2014 [24]	Korea	OP	1528	≤18yr	NPA	ARI	PCR	HBoV-1
Akhras 2010 [25]	US	ORT	256	<18yr	NPS	ARI	DFA, VC, PCR	RSV, HMPV
Ali 2010 [26]	Jordan	OP	728	<5yr	NS, TS	ARI	PCR	HMPV
Annamalay 2016 [27]	MZ	OP	277	≤10yr	NPA	RTI	PCR	HRV
Bhandary 2016 [28]	India	CS	100	≤5yr	NPA	RTIs	DFA	RSV
Broor 2014 [29]	US	OP	245	<5yr	NS, TS	ARI	PCR	IV A/B, RSV
Bryant 2010 [30]	AU	OP	446	≤16yr	NPA, NS, TS	ILI	DFA, PCR	IVA
Carballal 2002 [31]	AT	ORT	168	<2yr	NPA	Acute LRTI	IFA, VC	HAdV
Chang 2012 [32]	US	OP and CC	5066	≤18yr	NS	ILI	PCR	IVA
Chano 2005 [33]	Canada	CC	1132	≤18yr	NPA, BAL, ETA	RTI	DFA, VC EIA, PCR	HMPV
Chen 2010 [34]	China	OP	6296	≤18yr	NPA	Acute LRTI	PCR	RSV, HMPV
Cuevas 2003 [35]	Brazil	OP	111	<3yr	NS	Acute LRTI	PCR	RSV, HMPV
Esposito 2016 [36]	Italy	OP	307	≤18yr	NS	RTI	PCR	HAdV

Fairchok 2010 [37]	US	Cohort	318	≤30m	NS	RTI	PCR	IVA
Farng 2002 [38]	China	ORT	48	≤18yr	TS, serum	PNA	IFA	HAdV
Fischer 2013 [39]	GU	OP	2413	<5yr	NPS, OPS	ARI	PCR	RSV
Flores 2004 [40]	Portugal	OP	225	<3yr	NS	Acute BCL	PCR	RSV
Giamberardin 2016 [41]	Brazil	CS	250	24m-59m	NS, OPS	RTI, asthma	PCR	IV A/B, HRV, HAdV, HPIV, HCoV
Halasa, 2015 [42]	Jordan	OP	3173	<2yr	NS, TS	RTI, others	PCR	RSV
Hite 2007 [43]	UK	CC	411	≤18yr	NS	ILI	RT, VC	IV A/B
Hombrouck 2012 [44]	Belgium	OP	139	<5yr	NPS,TS	ILI	PCR	IVA, RSV, HRV, HMPV, HPIV
Hsieh 2014 [45]	China	OP	1062	≤18yr	serum	Flu season	Ab	IVA
Huai 2017 [46]	China	OP	14479	<15yr	NS	SARI	PCR	IVA/B
Jevsnik 2012 [47]	Slovenia	OP	741	<6yr	NPS, TS, TA, BAL, sputum	ARI	PCR	HCoV
Jin 2010 [48]	China	OP	645	<16yr	NPA	ARI	PCR	HCoV
Khamis 2012 [49]	Oman	OP	259	≤5yr	RS	RTI	PCR	RSV
Kuo 2011 [50]	China	CC	308	≤18yr	NPS, TS	ILI	RT, PCR	IVA
Lamarao 2012 [51]	Brazil	CS	1214	≤18yr	NPS	CAP	DFA, PCR	RSV
Landa-Cardena 2012 [52]	Mexico	CS	124	<6yr	NS	RTI	PCR	HRV

Leung 2009 [53]	China	OP and OR	1981	<18yr	NPA	ARI	IFA, PCR	HCoV
Martin 2015 [54]	Canada	OP	219	≤2yr	Oral fluid	HHP-6 history	PCR	HBoV-1
Moreno-Valencia 2015 [55]	Mexico	OP	432	<12yr	NPS	ARI	PCR	IVA, RSV, HRV, HMPV, HPIV, HAdV
Nitsch-Osuch 2013 [56]	Poland	OP	59	≤59m	NS, PS	ILI	RT, PCR	IV A/B
Nokes 2009 [57]	Kenya	OP	6026	1d-59m	NS	PNA	DFA	RSV
Nyawanda 2016 [58]	Kenya	OP	4714	<5yr	NPS, OPS	ARI	PCR	RSV
Pecchini 2008 [59]	Brazil	OP	455	<5yr	NPS	Acute LRTI	IFA	RSV
Pierangeli 2012 [60]	Italy	OP	231	≤16yr	PS, NW	ILI	PCR	IVA, RSV, HRV
Ramagopal 2016 [61]	India	OP	80	1m-3yr	NPS	BCL	PCR	RSV
Saha 2010 [62]	India	OP	69	10m-12yr	NS, TS	Acute FRI	PCR	IVA
Schuster 2015 [63]	Jordan	OP	3175	<2yr	NS, TS	TRI, AE, CF, FS	PCR	HMPV
Smit 2012 [64]	NL	OP	423	≤17yr	OPS, NW	ILI	PCR	IVA
Smuts 2011 [65]	South Africa	OP	220	2m-5yr	NS	cough, DB, wheez-ing	PCR	HRV
Tresoldi 2011 [66]	Brazil	Cohort	61	≤18yr	NPS, PS	ILI	PCR	IVA
von Linstow 2004 [67]	Denmark	OP	374	≤2yr	NPS	TRI	IFA, EIFA, PCR	RSV, HMPV

Weigl 2003 [68]	Germany	CC	1316	≤2yr	NPA	LRTI	PCR	RSV
Yan 2017 [69]	China	OP	387	8d-15yr	NPA	Acute LRTI	PCR	RSV, HMPV
Zimmerman 2014 [70]	US	CC	662	<2yr	NS, OPS	URTI	PCR	IV A/B, RSV, HRV, HMPV, HCoV

Ab: antibody, AE: asthma exacerbation, ARI: acute respiratory infection, AT: Argentina, AU: Australia, BALF: bronchoalveolar lavage fluid, BCL: bronchiolitis, CAP: community-acquired pneumonia, CC: case-control, CF: cystic fibrosis, CS: cross-sectional, DB: difficulty breathing, DFA: direct immunofluorescence assay, EIA: enzyme immunoassay, EIFA: enzyme immunofluorescence assay, ETA: endotracheal aspirate, IV: influenza virus, IVA: influenza A virus, FS: febrile seizure, FRI: febrile respiratory illness, GU: Guatemala, HHP-6: human herpesvirus 6, IFA: (indirect) immunofluorescence assay, ILI: Influenza-like illness, LRTI: lower respiratory tract infection, MZ: Mozambique, NL: Netherlands, NPA: nasopharyngeal aspirate, NPS: nasopharyngeal swab, NS: nasal swab/secretion, NW: nasal washing, OP: observational prospective, OPS: oropharyngeal swab, ORT: observational retrospective, PC: prospective cohort, PNA: pneumonia, PS: pharyngeal swab, RS: respiratory sample, SARI: severe acute respiratory infection, RT: rapid test, RTI: respiratory tract infection, TA: tracheal aspirate, TS: throat swab; URTI: upper respiratory tract infection, VC: virus culture.

8.5.2 Meta-analysis of literature review data

Meta-analysis of the published literature suggested several significant positive or negative associations between individual clinical features and different types of respiratory viral infections **(Figure 7)**.

In clinical practice, fever and wheezing/LRTI are often viewed as a "typical" hallmark of IV and RSV infections, respectively [53]. Thus these clinical associations were explored exhaustively in the published literature. It is worth noting that the individual sizes of pooled studies for IV and RSV were highest (N=24661 and N=29426, respectively) [53].

Indeed, fever was the feature most strongly associated with IV (pOR=3.0; 95%CI=2.0-4.3; I^2= 66%) (**Figure 8a**). As evident from meta-analysis of the individual literature dataset [53], most studies agreed on a *positive correlation*, with one exception (Tresoldi et al. 2011) [138] (**Figure 8a**). The significant association between wheezing/LRTI and RSV infection (pOR=2.2; 95%CI=1.7-2.8; I^2= 86%) was also identified by meta-analysis (**Figure 8b**). In-depth analysis of individual studies revealed positive associations for most RSV studies, with two exceptions

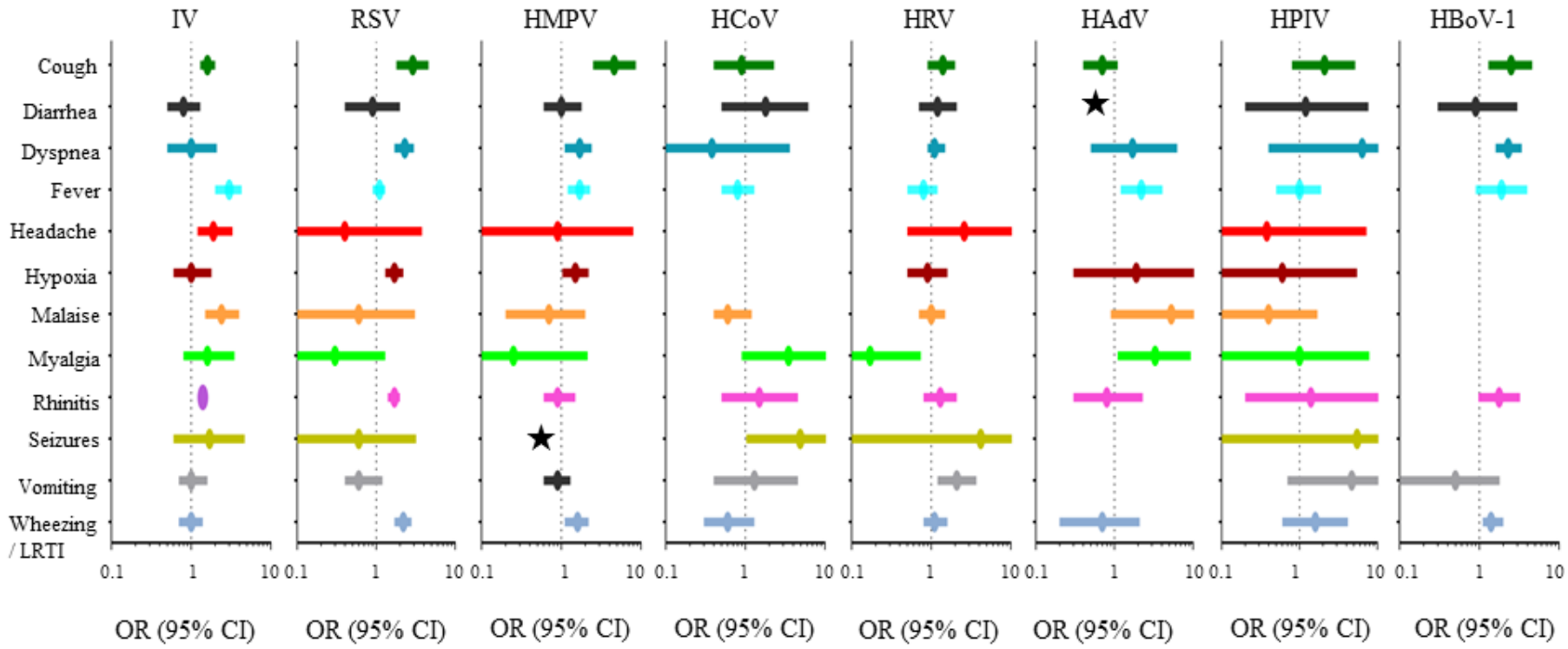
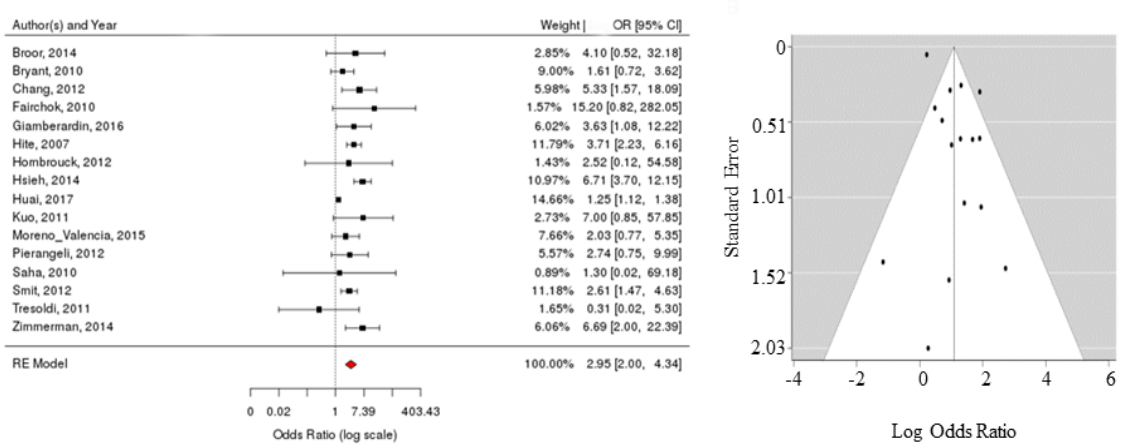


Figure 7: Summary of all statistically significant ($p<0.05$) features identified by the literature review dataset [53]. ★: OR values of seizures associated with HMPV and diarrhea associated with HAdV were estimated to be 16.6 and 14.4, respectively. Color legend: Blue: Altered/LOC, light yellow: Anorexia/DF, violet: Apnea, grass-green: Conjunctivitis, light green: Cough, yellow: Diarrhea, Peacock green: Dyspnea, cyan: Fever, Red: Headache, brown: Hypoxia, orange: Malaise, bottle-green: Myalgia, light blue: Rash, pink: Rhinitis, yellowish green: Seizures, fuchsia: Sore throat, light violet: URTI, grey: Vomiting, Cerulean blue: Wheezing/LRTI.

(Cuevas et al. 2013 and Halasa et al. 2015) [108, 113]. It is worth noting that publication bias was significant for RSV studies but not for IV studies (**Figures 8a and 8b**).

However, associations with fever or wheezing/LRTI are neither unique to IV nor to RSV (**Figure 8**). In addition to IV, fever was also significantly linked to HMPV (pOR=1.7; 95%CI=1.2-2.3; I^2= 45%) (**Figure 9a**) and HAdV infections (pOR=2.2; 95%CI =1.2-4.1; I^2= 11%) (**Figure 9b**) [53]. Wheezing/LRTI was correlated not only to RSV infections, but also to HMPV (pOR=1.6; 95%CI=1.1-2.2; I^2= 54%) (**Figure 9c**), and HBoV-1 infections (pOR=1.4; 95%CI=1.1-2.0; I^2= 0%) (**Figure 9d**) [53]. It is worth noting that significant publication bias was observed in the relationship between wheezing/LRTI and HMPV but none for the

a



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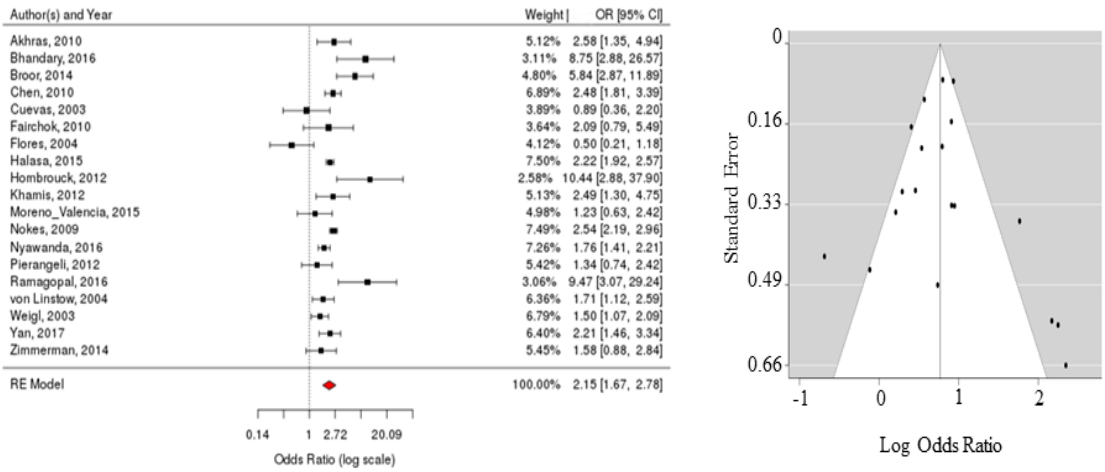
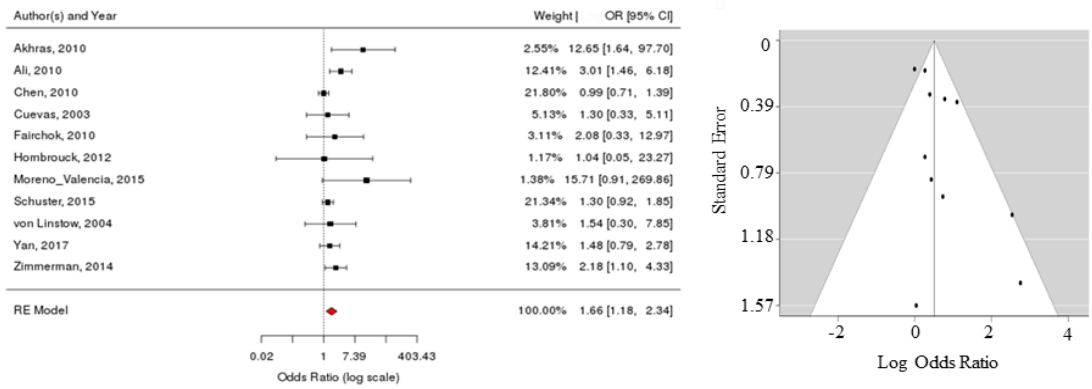


Figure 8: Forest plots (left) and funnel plots (right) of (a) fever associated with IV and (b) wheezing/LRTI associated with RSV [53]. In funnel plots, a symmetrical plot indicates no publication bias and an asymmetrical plot indicates publication bias.

a



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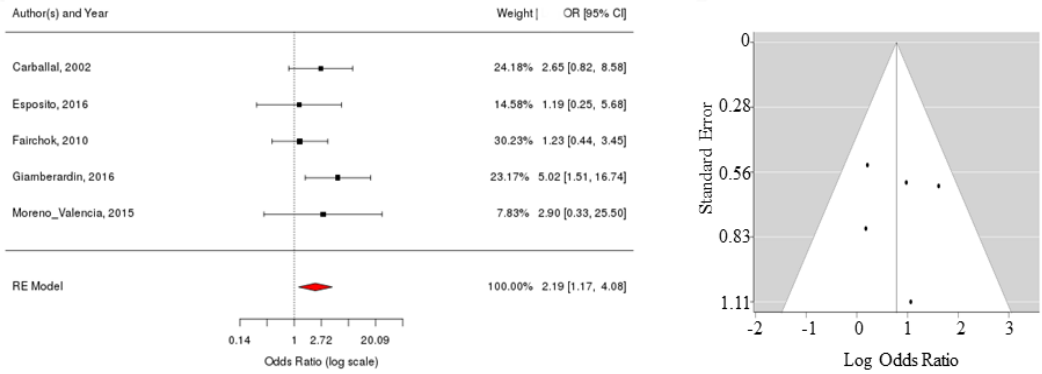

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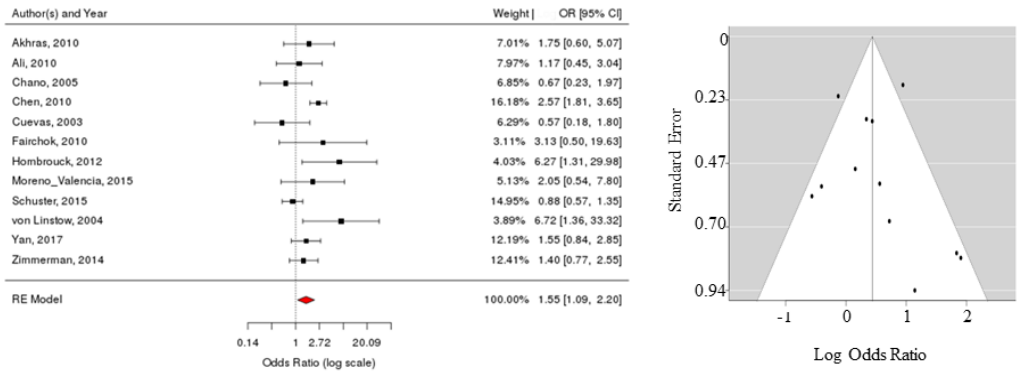

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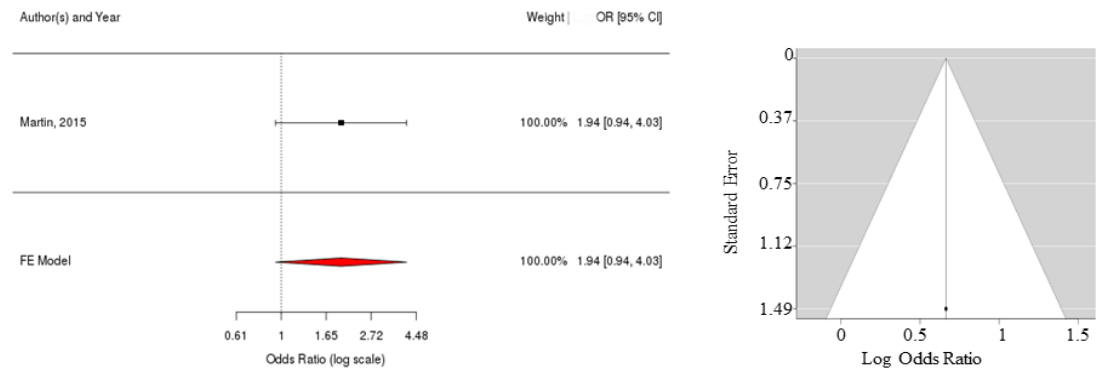

Figure 9: Forest plots (left) and funnel plots (right) of fever associated with (a) HMPV as well as (b) HAdV, and wheezing/LRTI associated with (c) HMPV as well as (d) HBoV-1. In funnel plots, a symmetrical plot indicates no publication bias and asymmetrical plot indicates publication bias.

relationship between fever and HMPV or HAdV, between wheezing/LRTI and HBoV-1 (**Figure 10**). It is worth noting that only one study was included for association between HBoV-1 and wheezing/LRTI.

Moreover, additional signs and symptoms were significantly linked to IV, including malaise (pOR=2.4; 95%CI=1.5-4.0; I^2=48%), headache (pOR=1.9; 95%CI=1.2-3.3; I^2=76%), cough (pOR=1.6; 95%CI=1.3-2.0; I^2=19%) and rhinitis (pOR=1.4; 95%CI=1.3-1.6; I^2=0%) **(Figure 7)** [53]. It is worth noting that positive evidence of publication bias was observed for malaise and headache but not for cough and rhinitis. The overview of all significant signs and symptoms in **Figure 7** suggested that cough and dyspnea were not only most strongly associated with RSV (pOR_{cough}=2.9; 95%CI=1.8-4.6; I^2= 77% and $pOR_{dyspnea}$=2.3; 95%CI=1.7-3.0; I^2= 84%), but were also shared by HMPV (pOR_{cough}=4.6; 95%CI=2.5-8.6; I^2=18% and $pOR_{dyspnea}$=1.7; 95%CI=1.1-2.4; I^2= 39%) and HBoV-1 infection (pOR_{cough}=2.5; 95%CI=1.3-4.6; I^2=0 and $pOR_{dyspnea}$=2.3; 95%CI=1.6-3.4; I^2=0) [53]. Cough was also associated with IV infection. There were publication biases between cough/RSV and dyspnea/HMPV.

8.5.3 Same and new association revealed in the inception cohort dataset

The same clinical features used in the literature review dataset (LIT) were now tested in the inception cohort dataset (COH) **(Table 13).** The first noticeable discrepancy was that narrower confidence intervals of associations were identified in the COH dataset than those in the LIT dataset. COH data confirmed published literature trends, usually with higher OR and higher confidence levels (i.e. narrower 95% CI) in spite of a smaller sample size. Exceptions included malaise/IV, fever/HMPV, vomiting/HRV, and malaise/HAdV, which could not be confirmed in the COH dataset [53].

Quite a few new positive as well as negative associations were identified in the COH dataset, which were not previously revealed by the meta-analysis in LIT dataset [53]. For instances, IV was associated with myalgia (OR 3.1; 95%CI=2.3-4.3) and sore throat (OR=1.8; 95%CI=1.5-2.1). Wheezing/LRTI (OR=0.4; 95%CI=0.3-0.5), hypoxia (OR=0.4; 95%CI=0.4-0.6), dyspnea (OR=0.5; 95%CI=0.4-0.6), rash and diarrhea (both OR=0.7; 95%CI =0.6-0.9) were negatively correlated to IV in the COH dataset.

As RSV infection for another example, difficulty feeding (DF) and apnea were positively correlated to RSV infection (OR=1.6; 95%CI=1.4-1.8 and OR=1.5; 95%CI=1.1-2.1, respectively). Additional *negative* associations were also identified for RSV, including fever

(OR=0.5; 95%CI=0.4-0.6) headache (OR=0.2; 95%CI=0.1-0.2), myalgia (OR=0.2; 95%CI=0.1-0.3), seizures (OR=0.4; 95%CI=0.3-0.5), rash (OR=0.8; 95%CI=0.6-0.9) and sore throat (OR=0.8; 95%CI=0.7-0.9) [53]. It is worth noting that headache was positively linked to IV but negatively linked to all other seven respiratory viral infections (RSV, HMPV, HRV, HCoV, HAdV, HPIV and HBoV).

Table 13: Comparison between literature review (LIT; pOR) and cohort data (COH; OR) [53].

Clinical Features	LIT	COH	LIT	COH	LIT	COH	LIT	COH	LIT	COH	LIT	COH	LIT	COH	LIT	COH
	IV		RSV		HMPV		HCoV		HRV		HAdV		HPIV		HBoV-1	
Altered/LOC	1.1 (0.4, 3.5)	1.2 (1.05, 1.4)	0.6 (0.2, 2.0)	1.0 (0.8, 1.1)	1.8 (0.2, 19.5)	0.9 (0.7, 1.2)		1.0 (0.8, 1.2)	1.5 (0.1, 15.7)	0.9 (0.8, 1.03)		0.9 (0.8,1.1)	0.7 (0.0, 15.6)	1.0 (0.8, 1.2)		1.0 (0.8, 1.1)
Anorexia/DF	1.4 (0.9, 2.3)	0.9 (0.8, 1.1)	1.4 (0.96, 1.9)	1.6 (1.4, 1.8)	0.9 (0.6, 1.2)	1.4 (1.1, 1.8)		0.9 (0.7, 1.1)	0.9 (0.2, 3.0)	0.9 (0.8, 1.03)		1.2 (1.05, 1.5)	1.1 (0.3, 4.5)	0.9 (0.7, 1.1)		1.1 (0.99, 1.3)
Apnea	0.5 (0.2, 1.7)	0.8 (0.5, 1.2)	0.3 (0.0, 2.4)	1.5 (1.1, 2.1)	2.1 (0.4, 13.3)	0.8 (0.4, 1.4)		0.8 (0.4, 1.5)		1.5 (1.1, 1.9)	1.9 (0.5, 7.3)	0.4 (0.2, 0.8)		1.6 (1.02, 2.4)		1.3 (0.9, 1.7)
Conjunctivitis	1.6 (0.5, 5.3)	1.0 (0.8, 1.4)	1.6 (0.2, 14.1)	0.9 (0.7, 1.1)	1.2 (0.2, 7.6)	0.7 (0.4, 1.1)	0.7 (0.1, 5.1)	1.7 (1.2, 2.4)	0.3 (0.0, 5.4)	1.2 (0.96, 1.4)	1.4 (0.5, 4.0)	1.4 (1.1, 1.9)	2.2 (0.4, 13.1)	1.3 (0.9, 1.8)		0.7 (0.5, 0.9)
Cough	1.6 (1.3, 2.0)	2.0 (1.6, 2.4)	2.9 (1.8, 4.6)	6.1 (4.7, 8.0)	4.6 (2.5, 8.6)	5.4 (3.3, 9.4)	0.9 (0.4, 2.3)	1.3 (0.97, 1.8)	1.4 (0.9, 2.0)	1.2 (1.02, 1.4)	0.7 (0.4,1.1)	0.9 (0.7,1.1)	2.1 (0.8, 5.2)	2.2 (1.7, 3.1)	2.5 (1.3, 4.6)	1.3 (1.1, 1.6)
Diarrhea	0.8 (0.5, 1.3)	0.7 (0.6, 0.9)	0.9 (0.4, 2.0)	1.1 (0.9, 1.3)	1.0 (0.6, 1.8)	1.0 (0.7, 1.4)	1.8 (0.5, 6.2)	0.9 (0.6, 1.3)	1.2 (0.7, 2.1)	0.9 (0.8, 1.1)	14.4 (2.5, 82.1)	1.4 (1.1, 1.7)	1.2 (0.2, 7.7)	1.0 (0.8, 1.4)	0.9 (0.3, 3.0)	1.0 (0.8, 1.2)
Dyspnea	1.0 (0.5, 2.1)	0.5 (0.4, 0.6)	2.3 (1.7, 3.0)	3.2 (2.7, 3.7)	1.7 (1.1, 2.4)	1.6 (1.3, 2.1)	0.4 (0.0, 3.6)	1.1 (0.9, 1.4)	1.1 (0.9, 1.5)	1.5 (1.4, 1.7)	1.7 (0.5, 6.3)	0.7 (0.6, 0.8)	6.4 (0.4, 112.5)	1.4 (1.1, 1.7)	2.3 (1.6, 3.4)	1.3 (1.1, 1.5)
Fever	3.0 (2.0, 4.3)	4.3 (2.8, 7.0)	1.1 (0.9, 1.3)	0.5 (0.4, 0.6)	1.7 (1.2, 2.3)	1.2 (0.8, 1.9)	0.8 (0.5, 1.3)	0.7 (0.5, 0.95)	0.8 (0.5, 1.2)	0.8 (0.6, 0.9)	2.2 (1.2, 4.1)	2.2 (1.5, 3.2)	1.0 (0.5, 1.9)	1.0 (0.7, 1.4)	1.9 (0.9, 4.0)	1.0 (0.8, 1.3)
Headache	1.9 (1.2, 3.3)	2.7 (2.2, 3.3)	0.4 (0.1, 3.8)	0.2 (0.1, 0.2)	0.9 (0.1, 8.0)	0.5 (0.3, 0.8)		0.4 (0.2, 0.7)	2.6 (0.5, 12.2)	0.5 (0.4, 0.7)		0.6 (0.4, 0.8)	0.4 (0.0, 7.3)	0.5 (0.3, 0.8)		0.5 (0.4, 0.7)
Hypoxia	1.0 (0.6, 1.8)	0.4 (0.4, 0.6)	1.7 (1.3, 2.2)	2.1 (1.8, 2.4)	1.5 (1.03, 2.2)	1.4 (1.03, 1.8)		0.9 (0.7, 1.2)	0.9 (0.5, 1.6)	1.4 (1.2, 1.5)	1.9 (0.3, 10.9)	0.6 (0.5, 0.7)	0.6 (0.1, 5.5)	1.1 (0.9, 1.4)		1.1 (0.9, 1.3)
Malaise	2.4 (1.5, 4.0)	0.9 (0.8, 1.1)	0.6 (0.1, 3.1)	1.2 (1.1, 1.4)	0.7 (0.2, 2.0)	1.2 (0.9, 1.5)	0.6 (0.4, 1.2)	0.9 (0.7, 1.2)	1.0 (0.7, 1.5)	1.0 (0.9, 1.1)	5.3 (0.9, 30.2)	0.8 (0.6, 0.9)	0.4 (0.1, 1.7)	1.0 (0.8, 1.2)		1.1 (0.9, 1.2)
Myalgia	1.6 (0.8, 3.5)	3.1(2.3, 4.3)	0.3 (0.1, 1.3)	0.2 (0.1, 0.3)	0.3 (0.0, 2.1)	1.2 (0.6, 2.2)	3.5 (0.9, 13.6)	0.4 (0.1, 0.99)	0.2 (0.0, 0.7)	0.6 (0.4, 0.9)	3.3 (1.1, 9.4)	0.7 (0.4,1.1)	1.0 (0.1, 7.9)	0.4 (0.1, 0.8)		0.6 (0.4, 0.9)
Rash		0.7 (0.6, 0.9)	0.4 (0.1, 3.0)	0.8 (0.6, 0.9)	1.2 (0.1, 9.9)	0.6 (0.4, 0.95)		0.8 (0.6, 1.2)		0.9 (0.8, 1.1)		1.2 (0.9,1.5)		1.1 (0.8, 1.5)	1.2 (0.3, 5.2)	1.1 (0.9, 1.3)
Rhinitis	1.4 (1.3, 1.6)	1.6 (1.3, 1.9)	1.7 (1.4, 2.0)	2.0 (1.7, 2.4)	0.9 (0.6, 1.5)	1.9 (1.4, 2.6)	1.5 (0.5, 4.6)	1.2 (0.9, 1.6)	1.3 (0.8, 2.1)	1.6 (1.4, 1.9)	0.8 (0.3, 2.3)	1.8 (1.5, 2.3)	1.4 (0.2, 10.5)	1.0 (0.8, 1.3)	1.8 (0.97, 3.2)	1.1 (0.9, 1.2)
Seizures	1.7 (0.6, 4.7)	1.3 (0.99, 1.6)	0.6 (0.1, 3.2)	0.4 (0.3, 0.5)	16.6 (0.6, 438.1)	0.8 (0.5, 1.2)	4.9 (1.03, 23.0)	0.9 (0.6, 1.4)	4.2 (0.1, 222.1)	0.9 (0.7, 1.1)		1.4 (1.1, 1.8)	5.5 (0.1, 295.9)	1.0 (0.7, 1.4)		1.1 (0.9, 1.3)
Sore throat	1.4 (0.8, 2.4)	1.8 (1.5, 2.1)	0.7 (0.3, 1.9)	0.8 (0.7, 0.9)	0.7 (0.3, 2.1)	1.0 (0.8, 1.3)	0.7 (0.4, 1.2)	0.9 (0.7, 1.1)	0.7 (0.4, 1.1)	0.9 (0.8, 1.1)		1.9 (1.6, 2.3)		0.8 (0.6, 0.95)		1.1 (0.9, 1.2)
URTI	0.6 (0.2, 1.5)	1.4 (1.03, 2.0)	0.6 (0.3, 1.3)	1.1 (0.8, 1.4)	1.3 (0.7, 2.4)	1.0 (0.7, 1.7)	1.8 (0.1, 31.8)	0.9 (0.6, 1.4)	0.7 (0.2, 2.8)	1.2 (0.9, 1.5)		1.4 (0.96,2.0)	3.0 (0.3, 25.7)	1.0 (0.7, 1.6)		1.5 (1.1, 2.0)
Vomiting	1.0 (0.7, 1.6)	0.8 (0.7, 0.98)	0.6 (0.4, 1.2)	1.0 (0.9, 1.2)	0.9 (0.6, 1.3)	1.1 (0.8, 1.4)	1.3 (0.4, 4.6)	0.9 (0.7, 1.1)	2.1 (1.2, 3.7)	0.9 (0.8, 1.1)		1.1 (0.9,1.3)	4.7 (0.7, 33.0)	1.1 (0.9, 1.4)	0.5 (0.1, 1.8)	1.1 (0.9, 1.2)
Wheezing/LRTI	1.0 (0.7, 1.4)	0.4 (0.3, 0.5)	2.2 (1.7, 2.8)	6.2 (5.3, 7.3)	1.6 (1.1, 2.2)	2.9 (2.2, 3.9)	0.6 (0.3, 1.3)	1.1 (0.9, 1.4)	1.1 (0.8, 1.6)	1.4 (1.2, 1.6)	0.7 (0.2, 2.1)	0.7 (0.6, 0.8)	1.6 (0.6, 4.2)	1.2 (0.9, 1.4)	1.4 (1.1, 2.0)	1.3 (1.2, 1.5)
Case numbers	N=24661	N=6042	N=29426	N=6042	N=14010	N=6042	N=4597	N=6042	N=2653	N=6042	N=1523	N=6042	N=1139	N=6042	N=1747	N=6042

dark green color: positive agreement with statistically significant positive associations in both LIT and COH datasets.
dark red color: negative agreement with statistically significant negative associations in LIT and COH.
light green color: significant positive association in either LIT or COH, but not the other; light red color: significant negative association in either LIT or COH, but not the other;
grey color: borderline-significant associations (i.e. CI values close to 1).
N: Number of study subjects with diagnostic testing and clinical data.

In summary, both dataset analyses indicated that fever was significantly correlated to IV and HAdV infections, whereas the presence of fever did not exclude any of the other types of viral infection. HAdV infections were also associated with diarrhea [53]. Cough was most likely present in patients with IV, RSV, HMPV, and HBoV-1 infections, but also shared by other types of viral respiratory infections [53]. Wheezing was most strongly associated with RSV, HMPV and HBoV-1 infections. Wheezing was less likely to be observed in IV and HAdV infections in the COH dataset, whereas the LIT dataset indicated inconclusive results in this regard [53]. HCoV suggested no agreements between LIT and COH datasets, with no data available in the literatures reporting a number of non-respiratory symptoms [53]. The relationship between myalgia and HRV infection was the only negative association confirmed in both LIT and COH datasets [53].

8.6 Decision tree models as tools for virus-feature analyses

An individual clinical feature does not distinguish one virus from the other viruses using traditional regression methodology, but several significant associations with a specific type of viral infection were revealed. Thus, separate novel conditional inference decision tree (CIDT) models were developed for eight respiratory viral infections: (1) combination of clinical features (CIDT model 1), (2) clinical features and risk factors (CIDT model 2), and (3) clinical features, risk factors and seasonal pattern (CIDT model 3). Referenced to cost-effectiveness analysis for pediatric IV infection [143, 144], the low, moderate and high risks for possibility of positive IV and other viral infections were assessed to be <18%, 18%-60% and >60%, respectively [143, 144]. Therefore, the probability of specific positive viral infection in each terminal node for CIDT models was calculated for the low-risk group, intermediate-risk group, and high-risk group.

8.6.1 Influenza virus

8.6.1.1 CIDT model based on clinical features (model 1)

The CIDT analysis for children with IV infection based on the reference standard of clinical features only is shown in **Figure 10**. The training data consists of 1352 patients (22.4% of 6042 patients). The final decision tree algorithm covered six clinical features: LRTI, cough, fever, headache, seizure and need for hospitalization.

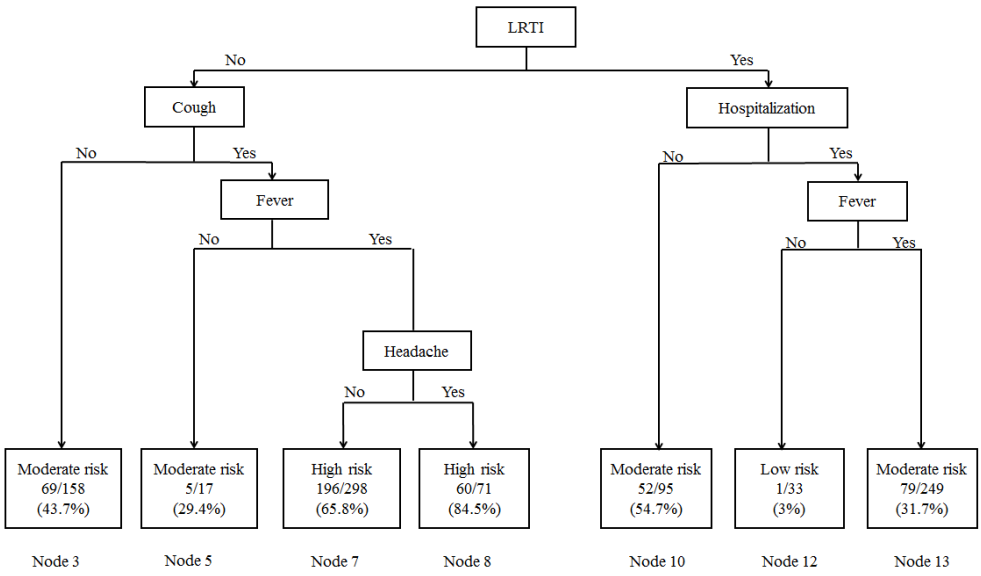

Figure 10: A conditional inference decision tree (CIDT) model for predicting IV based on clinical features (IV CIDT model 1)

Within the initial study subpopulation, there were cases with or without LRTI. For cases without LRTI, subsequent recursive partitioning suggested moderate-risk subpopulations: (1) children presenting *without* cough (43.7%, node 3), and (2) children presenting with cough but *without* fever (29.4%, node 5). In addition, two high-risk subgroups were also revealed: (1) children presenting with cough, fever and headache (84.5%, node 8), and (2) children presenting with cough and fever but *without* headache (65.8%, node 7). Presence of LRTI, need for hospitalization was the second predictor. Subsequent recursive partitioning indicated a low-risk subpopulation of IV infection: hospitalized patients presenting with LRTI but *without* fever (3%, node 12). Either hospitalized patients presenting with LRTI and fever (31.7%, node 13) or outpatients presenting with LRTI (54.7%, node 10) were at moderate risk of IV infection. It is worth noting that these children who did not present with either LRTI or cough were half as likely to get an IV infection (43.7%, node 3).

Using these predictors, the model (IV CIDT model 1) yielded a predictive accuracy of 65.7% (95% CI= 61%-70.1%) based on clinical features, i.e., it correctly predicted either a positive or negative diagnosis of control 66% of the time. The area under the receiver operating characteristic curve (AUC) for this model was 0.687. The sensitivity and specificity was 60.8% (95% CI= 56.1%-65.3%) and 70.6% (95% CI= 66.1%-74.7%), respectively.

8.6.1.2 CIDT model based on clinical features and risk factors (model 2)

The CIDT model (model 2) for predicting IV infection based on clinical features and risk factors (including children younger than 2 years and all underlying medical conditions shown in **Table 7**) are shown in **Figure 11**. The training data consists of 1352 patients. The final decision tree algorithm covered six clinical variables: children younger than 2 years old, LRTI, cough, need for hospitalization, fever and dyspnea.

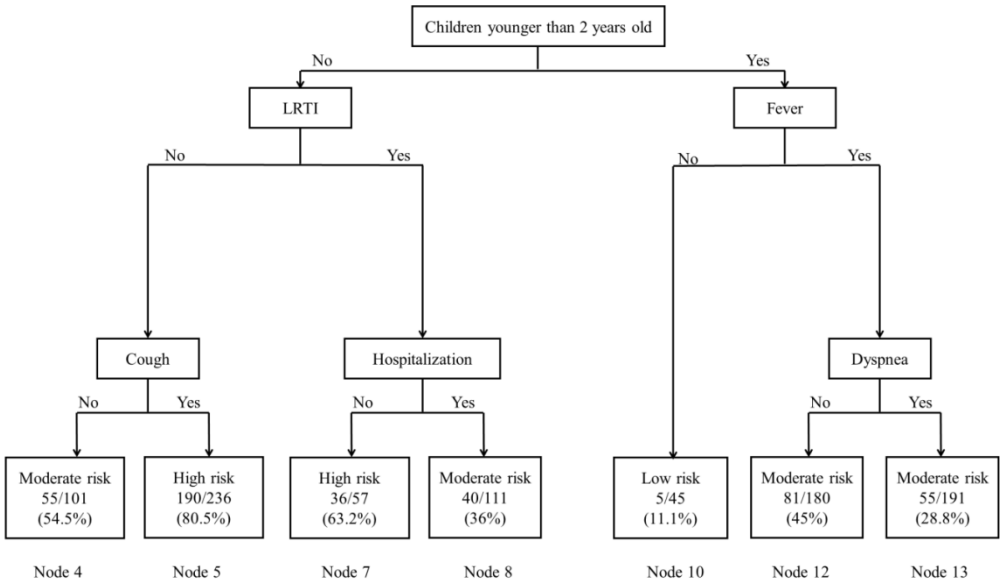

Figure 11: A conditional inference decision tree (CIDT) model for predicting IV based on clinical features and item risks (IV CIDT model 2)

The variable of children younger than 2 years old was the first predictor. The low-risk subgroup of IV diagnosis was identified in children younger than 2 years old with *absence* of fever (11.1%, node 10), whereas children with fever but *without* dyspnea (45%, node 12), and (3) children with fever and dyspnea (28.8%, node 13) were at moderate risk of IV. Other two moderate-risk subgroups were identified among children≥2 years old: (1) all children presenting *without* LRTI and cough (54.5%, node 4), and (2) hospitalized children presenting with LRTI (36%, node 8). In addition, high-risk subpopulations were either for children≥2 years old presenting with cough but *without* LRTI (80.5%, node 5) or for pediatric outpatients ≥2 years old with LRTI (63.2%, node 7).

The predicative accuracy, sensitivity and specificity for this mode were 68% (95% CI= 63.4%-72.2%), 71.9% (95% CI= 67.5%-75.9%) and 64% (95% CI= 59.4%-68.4%), respectively. The AUC was 0.738.

8.6.1.3 CIDT model based on clinical features, risk factors and seasonal pattern (model 3)

The final conditional inference decision tree algorithm covered five clinical variables: three levels (low, moderate and high) of seasonal pattern, infants younger than 2 years old and fever (**Figure 12**). The training data covered 1352 patients. The level of IV activity was generally observed to be moderate to high from December to March of the following year, with a peak in January or February, while it remained low from April to November.

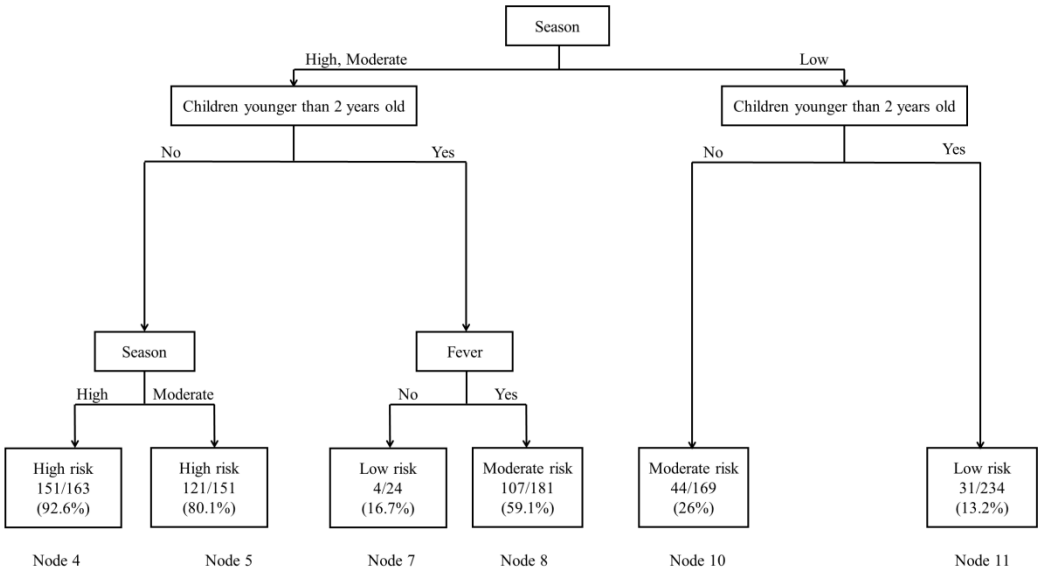

Figure 12: A conditional inference decision tree (CIDT) model for predicting IV based on clinical features, risk factors and seasonal pattern (IV CIDT model 3)

All the children ≥2 years old were at high risk of IV infection annually during December to March of the following year (node 4: 92.6% and node 5: 80.1%, respectively). Each year from December to March of the following year, children less than 2 years old were at moderate risk if they presented with fever (59.1%, node 8) but were at low risk if without fever (16.7%, node 7). During low level of IV seasonal pattern (annually from April to November), children younger than 2 years old were less likely to get IV infection, whereas children ≥2 years old had moderate risk of IV infection.

The predictive accuracy, sensitivity and specificity of this model were 79.5% (95% CI= 75.5%-83.1%), 69.8% (95% CI= 65.3%-74%) and 89% (95% CI= 85.7%-91.6%), respectively. The AUC for this model was 0.857.

8.6.2 RSV

8.6.2.1 CIDT model based on clinical features (model 1)

The CIDT model showed that LRTI was an initial partitioning variable (**Figure 13**). The training data consisted of 2072 patients (34.3% of 6042 patients). The final decision tree algorithm included five clinical features: LRTI, cough, headache, seizure and rhinitis.

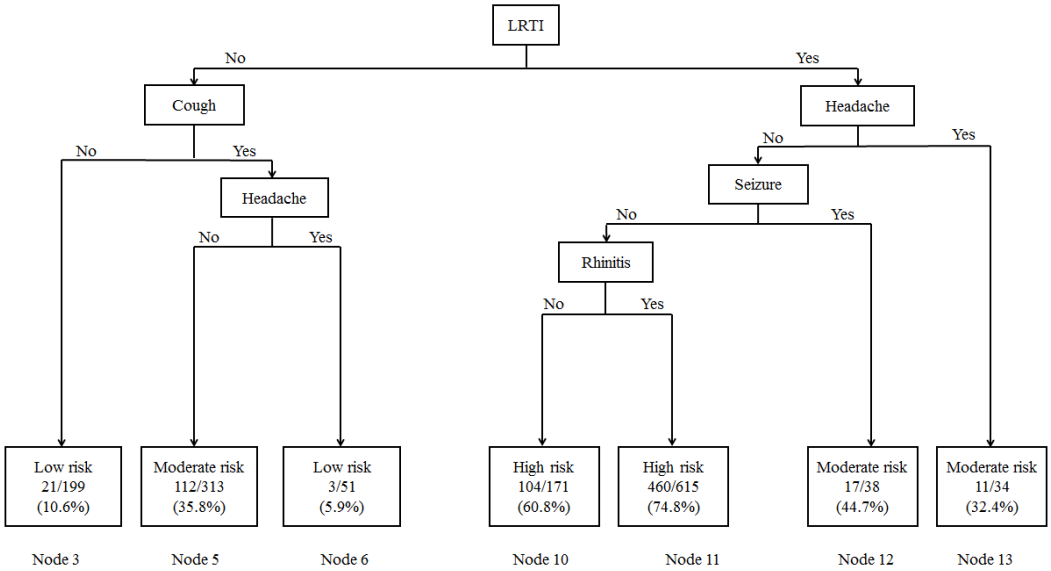

Figure 13: A conditional inference decision tree (CIDT) model for predicting RSV infection based on clinical features (RSV CIDT model 1)

Children presenting with LRTI but *without* headache and without seizure (regardless of rhinitis) had a high risk of positive RSV infections (node 11: 74.8% and node 10: 60.8%, respectively), whereas children with LRTI and combined with either seizure or headache were at moderate risk of RSV infection (node 12: 44.7% and node 13: 32.4, respectively). In contrast to children without LRTI, those who presented with cough and headache (5.9%, node 6) were at low risk of RSV infection, while whose manifesting cough but without headache were at moderate risk of RSV infection (35.8%, node 5). It is worth noting that in children presenting *without* LRTI or cough, RSV infection cannot be completely ruled out (10.6%, node 3).

The predicative accuracy, sensitivity and specificity for this mode were 69.6% (95% CI= 65.9%-73%), 66.5% (95% CI= 62.8%-70%) and 73.1% (95% CI= 69.5%-76.3%), respectively. The AUC was 0.745.

8.6.2.2 CIDT model based on clinical features and risk factors (model 2)

The CIDT model showed that LRTI was the first determinant, followed by cough, children younger than 2 years old, headache, and seizure (**Figure 14**). The training data consisted of 2072 patients.

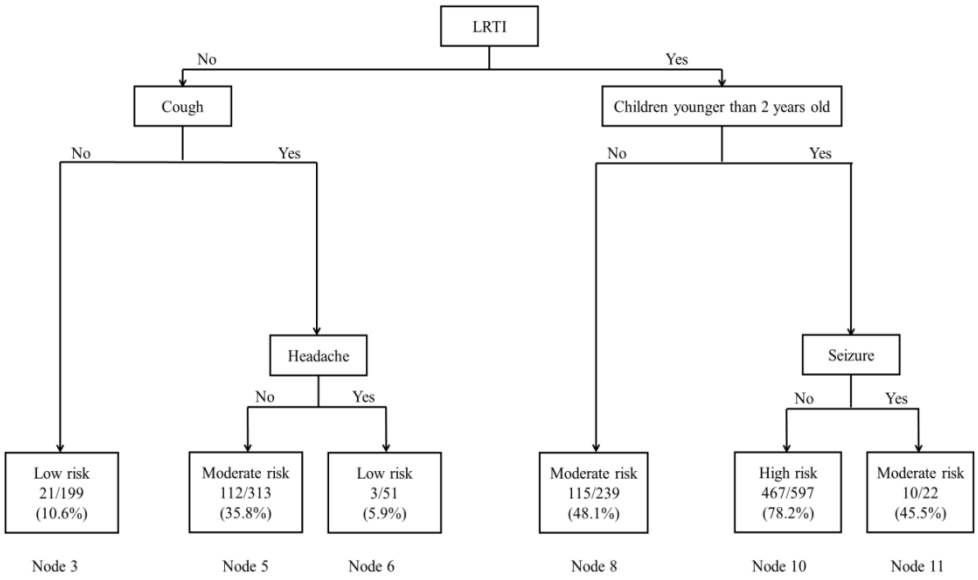

Figure 14: A conditional inference decision tree (CIDT) model for predicting RSV infection based on clinical features and risk factors (RSV CIDT model 2)

Children less than 2 years old presenting with LRTI but *without* seizure were at high risk of positive RSV infection (78.2%, node 10); whereas children with LRTI and seizure were at moderate risk (45.5%, node 11). In addition, moderate risk was also revealed among children aged more than 2 years presenting with LRTI (node 8: 48.1%) or all children with cough but *without* LRTI or headache (35.8%, node 5). It is worth noting that either children presenting with cough and with headache but *without* LRTI (5.9%, node 6) or those manifesting *without* LRTI or cough (10.6%, node 3) might be at low risk of RSV infection.

The predictive accuracy, sensitivity and specificity of this model were 70.2% (95% CI= 66.6%-73.6%), 80.5% (95% CI= 77.2%-83.3%) and 58.8% (95% CI= 54.9%-62.5%), respectively. The AUC for this model was 0.751.

8.6.2.3 CIDT model based on clinical features, risk factors and seasonal pattern (model 3)

RSV CIDT model 3 identified six variables: three different levels (low/moderate/high) of RSV seasonal activity, children younger than 2 years old, LRTI and cough (**Figure 15**). The training data were composed of 2072 patients. From November to April of the following year,

a moderate to high level of RSV circulation was observed each year, with a peak in December or February. In comparison, a low level of RSV circulation was generally identified between May and October.

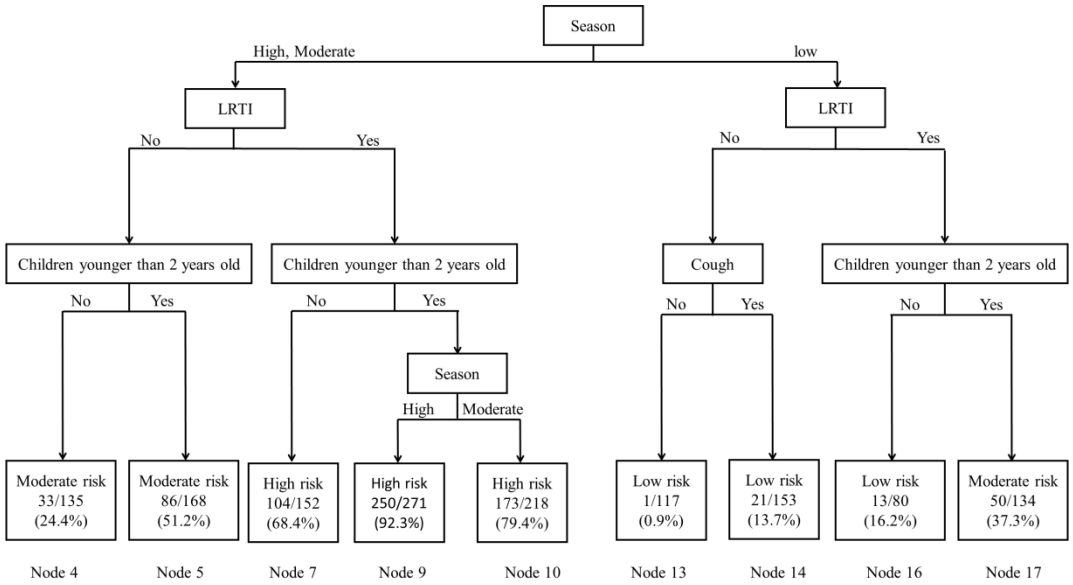

Figure 15: A conditional inference decision tree (CIDT) model for predicting RSV infection based on clinical features, risk factors and seasonal pattern (RSV CIDT model 3)

Within a moderate to high intensive of RSV season, all children with LRTI were at high risk of getting RSV infection. Among pediatric patients with LRTI, those under 2 years of age had a higher risk (92.3%, node 9 and 79.4%, node 10) than those ≥ 2 years of age (68.4%, node 7). The highest-risk population was these under two years of age in high level of RSV circulation (December or February) each year (92.3%, node 9). Although absence of LRTI (well-known "typical" or "perceived" feature of RSV), this pediatric population still had a moderate risk of RSV infection during moderate to high level of RSV circulation each year (November to April of the following year), regardless of the age of patients (24.4%, node 4 and 51.2%, node 5, respectively). In comparison to low level of RSV activity (annually between May and October), children aged younger than two years and presenting with LRTI were still at moderate risk (37.3%, node 17), requiring testing to confirm the RSV diagnosis. All the low-risk subpopulation was identified during low level of RSV activity among the following groups: (1) children aged ≥2 years manifesting with LRTI (16.2%, node 16), (2) all children presenting *without* LRTI regardless of whether or not cough was present (0.9%, node 13 and 13.7%, node 14). It is worth noting that RSV infection was unlikely if patients presented without LRTI and cough during low level of RSV activity (0.9%, node 13).

The predictive accuracy, sensitivity and specificity of this model were 75.9% (95% CI= 72.5%-79.1%), 69.6% (95% CI= 66%-73%) and 83% (95% CI= 79.9%-85.7%), respectively. The AUC for this model was 0.843.

8.6.3 HMPV

8.6.3.1 CIDT model based on clinical features (model 1)

The CIDT model (HMPV model 1) demonstrated that cough was significant when cases were *without* LRTI (**Appendix 1**). Pediatric cases with LRTI were at a high risk of HMPV-positive infection regardless of cough (61.2%, node 5). By contrast, pediatric patients presenting *without* LRTI but with cough were at moderate risk (40%, node 4).

The training data comprised 8.7% of 6073 patients or 528 patients. The AUC for training data was 0.61. The predictive accuracy, sensitivity and specificity at this cut-off value were 59.1% (95% CI= 51.2%-66.5%), 57.5% (95% CI= 49.6%-65.1%) and 60.5% (95% CI= 52.6%-67.9%), respectively.

8.6.3.2 CIDT model based on clinical features and risk factors (model 2)

When risk factors were added in a stepwise manner, this CIDT model (HMPV CIDT model 2) was identical only to the model of clinical features used (HMPV CIDT model 1): LRTI and cough were important predictors for HMPV-positive infection.

8.6.3.3 CIDT model based on clinical features, risk factors and seasonal pattern (model 3)

If clinical features, risk factors and seasonal pattern were added, the CIDT model (HMPV CIDT model 3) illustrated that the level of HMPV seasonal activity was the main determinant of possibility of getting HMPV infection, followed by cough and LRTI (**Appendix 2**). During medium-high level of HMPV seasonal pattern (winter-early spring), children with cough had a high risk of HMPV infection (75.9%, node 4), while those presenting without cough were still at 31.6% of likelihood of getting HMPV infection (31.6%, node 3). In the process of low level of HMPV circulation (between mid-spring and autumn), pediatric patients with LRTI were at moderate risk (30.4%, node 7); while those without LRTI were at low risk of HMPV infection (9.4%, node 6).

The training data included 528 patients. The AUC for training data was 0.742. The predictive accuracy, sensitivity and specificity of this validated model was 72.7% (95% CI= 65.2%-75.3%), 68.5% (95% CI= 52.6%-67.9%) and 76.5% (95% CI= 69.3%-82.5%), respectively.

8.6.4 HRV

8.6.4.1 CIDT model based on clinical features (model 1)

When introducing 22-item clinical features, the CIDT model demonstrated that LRTI was important when rhinitis was absent, and that headache was significant when rhinitis was present (**Appendix 3**). The children presenting either with rhinitis but without headache or presenting without rhinitis but with LRTI had a more than 50% likelihood for getting HRV infection (54.2%, node 6 and 50.4%, node 4, respectively). One third of these pediatric patients with both rhinitis and headache or presenting *without* rhinitis or LRTI were both at moderate-risk of HRV infection.

The training data consisted of 2706 patients or 44.5% of 6073 patients. The AUC for training data was 0.562. The low predictive accuracy (54.6%, 95% CI= 51.1%-58%) and sensitivity (25%, 95% CI= 22.1%-28.1%) were revealed at this threshold. Comparatively higher specificity (83.1%, 95% CI= 80.3%-85.5%) was identified.

8.6.4.2 CIDT model based on clinical features and risk factors (model 2)

When risk factors were introduced in a stepwise manner, the CIDT model (HRV CIDT model 2) was identical to the HRV CIDT model 1 based on clinical features, in which rhinitis, LRTI and headache were important predictors for a moderate risk of getting HRV infection.

8.6.4.3 CIDT model based on clinical features, risk factors and seasonal pattern (model 3)

When the seasonal pattern was introduced in a stepwise manner, the CIDT model (HRV CIDT model 3) yielded three significant predictors: headache, rhinitis and fever (**Appendix 4**). Of note, the variable of seasonal pattern was not included in the model. The training data were composed of 2706 patients. Children presenting *without* headache or fever but with rhinitis were at a 67.7% risk of getting HRV infection (node 5). Children presenting without headache but with rhinitis and fever (52.7%, node 6) and those with headache were both at moderate risk of HRV infection (33.5%, node 7). It is worth noting that children presenting *without* headache or rhinitis still had a 43.5% risk of getting HRV infection (node 3).

The AUC for training data was 0.56. Once again, the low predictive accuracy (56.3%, 95% CI= 52.8%-59.7%) and sensitivity (36.3%, 95% CI= 33.1%-39.8%) were identified at this cut-off point. Moderate specificity (75.6%, 95% CI= 72.5%-78.5%) was calculated.

8.6.5 HAdV

8.6.5.1 CIDT model based on clinical features (model 1)

The CIDT analysis using clinical features to predict HAdV infection is illustrated in **Appendix 5**. The training data consisted of 1142 patients. The initial predictor was LRTI. This CIDT model (HAdV CIDT model 1) revealed that pediatric patients presenting with LRTI and seizure had the highest risk of HAdV infection (84.2%, node 9). Around sixty percent of likelihood for HAdV was observed among either those who presented with LRTI and prolonged high fever (>40^{o}C) ≥ three days but with absence of seizure (60%, node 8) or those who were without LRTI but had fever (≥38^{o}C) (21.4%, node 4). More than one third of children presenting with LRTI but without seizure or prolonged high fever were at moderate risk of getting HAdV infection (34.9%, node 7). However, for more than one fifth of pediatric patients without LRTI or fever, a positive HAdV infection could not be ruled out (21.4%, node 3).

The AUC for training data was bad (0.52). Low predictive accuracy (51.9%, 95% CI= 46.4%-57.4%) and sensitivity (42.2%, 95% CI= 36.8%-47.7%) and moderate specificity (63%, 95% CI= 57.5%-68.2%) were noted.

8.6.5.2 CIDT model based on clinical features and risk factors (model 2)

The CIDT model (HAdV CIDT model 2) using clinical features and risk factors was identical to the model for clinical features only (HAdV CIDT model 1).

8.6.5.3 CIDT model based on clinical features, risk factors and seasonal pattern (model 3)

When the seasonal pattern was gradually introduced, the crucial factors of recursive partitioning were identified by rhinitis, LRTI and headache (**Appendix 6**). Similar to HRV model, the levels of HAdV seasonal pattern did not influence this model. The training data for this model included 1142 patients. Children with rhinitis but *without* LRTI or headache had a 66.5% risk of likelihood of HAdV infection (node 5). For those presenting with rhinitis and headache but without LRTI, and those with rhinitis and LRTI, both groups had a moderate

risk of HAdV infection. The latter group had the slightly higher risk (node 6: 35.9% versus node 7: 46.9%). However, more than one third of pediatric patients *without* rhinitis were likely to be diagnosed with HAdV infection (35.4%, node 2).

Compared to the HAdV CIDT model 1 and 2, the AUC (0.54) and predictive accuracy yielded by the HAdV CIDT model 3 (55.8%, 95% CI= 50.2%-61.2%) was somewhat improved but still low. By contrast, this model yielded moderate sensitivity (73.5%, 95% CI= 68.3%-78.1%) and low specificity (35.6%, 95% CI= 30.5%-41.1%).

8.6.6 HBoV-1

8.6.6.1 CIDT model based on clinical features (model 1)

When using 22-item clinical features, the CIDT model (HBoV-1 CIDT model 1) showed that Headache was only one important predictor for moderate-risk of getting HBoV-1 infection (**Appendix 7**). The training data for this model were composed of 1888 patients. This model yielded low comparative predictive capacity for positive HBoV-1 infection: AUC (0.52), predictive accuracy (52.4%, 95% CI= 48.2%-56.5%), sensitivity (10.7%, 95% CI= 8.4%-13.5%) and specificity (92.9%, 95% CI= 90.4%-94.7%).

8.6.6.2 CIDT model based on clinical features and risk factors (model 2)

Similar to models of HRV and HAdV when risk factors were gradually added, the corresponding CIDT model (HBoV-1 CIDT model 2) for predicting HBoV-1 infection was identical to the model only using clinical features (HBoV-1 CIDT model 1).

8.6.6.3 CIDT model based on clinical features, risk factors and seasonal pattern (model 3)

When seasonal pattern was gradually added, the first important predictor was the level of seasonal pattern, followed by children younger than 2 years old (**Appendix 8**). No clinical features were included into this CIDT model (HBoV-1 CIDT model 3). The training data still consisted of 1888 patients. Higher likelihood of HBoV-1 infection was revealed in moderate to high level of HBoV-1 activity (winter months) than in summer months when HBoV-1 activity is low. During low level of seasonal activity, children under 2 years of age might still be at moderate risk of HBoV-1 infection (36.1%, node 7), whereas children ≥ 2 years of age were at low risk (14.3%, node 6).

This model yielded moderate comparative predictive capacity for positive HBoV-1 infection: AUC (0.66), predictive accuracy (61.1%, 95% CI= 56.9%-65%), sensitivity (68.4%, 95% CI= 64.4%-72.1%) and specificity (53.9%, 95% CI= 49.8%-58%).

8.6.7 HCoV and HPIV

When introducing clinical features only or gradually adding risk factors, the CIDT models (CIDT model 1 and 2) demonstrate that there was no definite predictor to accurately predict positive HCoV or HPIV infection (figures not shown). The possibility of positivity or negativity for HCoV or HPIV was equal. The predictive accuracy, sensitivity and specificity for predicting HCoV were 45.8% (95% CI= 38%-53.7%), 100% and 0%, respectively. For HPIV, the predictive accuracy, sensitivity and specificity were 48.7% (95% CI= 42%-55.4%), 100% and 0%, respectively.

When seasonal patterns were gradually introduced, the level of seasonal pattern was the only one partitioning parameter to predict HCoV or HPIV infection (**Appendix 9**). Higher incidence of HCoV occurs in the community, higher likelihood to predict HCoV infection. However, this trend was not revealed for HPIV infection.

The HCoV CIDT model 3 (HCoV CIDT model 3) yielded 0.593 of AUC, 57.5% (95% CI= 49.9%-64.7%) of predictive accuracy, 25% (95% CI= 19%-32.1%) of sensitivity and 93.7% (95% CI= 88.9%-96.5%) of specificity, respectively. The CIDT model for HPIV (HPIV CIDT model 3) yielded predictive accuracy; sensitivity and specificity were 55.9% (95% CI= 49.4%-62.2%), 81.2% (95% CI= 75.6%-85.7%) and 29.5% (95% CI= 23.9%-35.7%), respectively.

In general, CIDT models for IV and RSV revealed a higher predictive capacity, whereas the remaining respiratory pathogens yielded a lower predictive capacity. The predictive values for each viral infection were low for variables that included clinical features only, but higher for clinical features plus risk factors, and highest for clinical features plus risk factors plus seasonality (**Figure 16**). Compared to IV CIDT model 1 and 2, IV CIDT model 3 yielded a high specificity of 89% and comparable sensitivity of 69.8%. Similarly, among RSV CIDT models, model 3 had the best predicting value of RSV infection with a specificity of 83% and sensitivity of 69.6%.

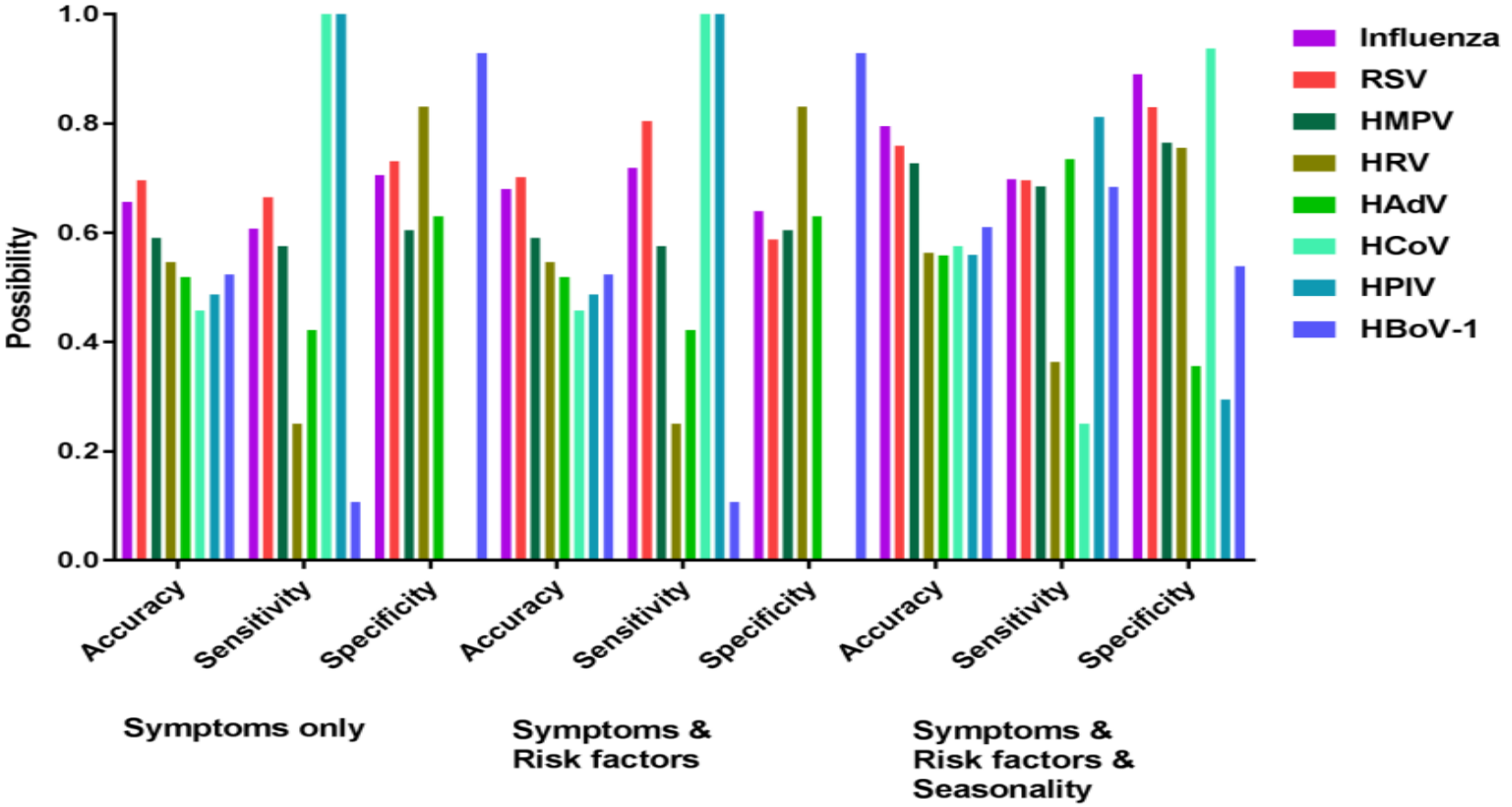

Figure 16: Predictive accuracy, sensitivity and specificity for the prediction of eight common respiratory viruses using the conditional inference decision tree (CIDT) model based on symptoms only, and gradually addition of risk factors and seasonality.

9 Discussion

9.1 Incidence of respiratory viruses in children with ILI

In this inception cohort study, 6042 children 0 to 19 years of age with ILI were prospectively enrolled from hospitals of the Charité Medical University in Berlin, Germany, from December 2009 to April 2015, and nasopharyngeal swabs were collected from each subject. The majority (57%) of patients were younger than two years old, suggesting that very young children are most affected. The same finding was confirmed by others[16, 145, 146]. For example, a pediatric inpatient study from Bonn, Germany, included 539 children 0 to 13 years old with ARI during November 2007 to December 2008, of which 60% of the patients were younger than two years [145]. Another recent European study enrolled 3199 pediatric patients under 16 years of age with ARI between September 2012 and August 2016 in France, and 73% of the affected patients were less than two years old [146].

Specimens were investigated for respiratory viruses, including IV, RSV, HMPV, HRV, HAdV, HCoV, HPIV and HBoV-1, by real-time PCR. Seventy percent were positive for at least one viral pathogen. Comparable viral incidence rates were reported by investigators in Germany and other European countries. For instance, a previous study analyzed nasal and pharyngeal swabs from children aged 0 to18 years were investigated for nine viruses (including IV, RSV, HMPV, HRV, HAdV, HBoV, HPIV [type 1, 2, and 3], HCoV [229E, OC43 and NL63] and enterovirus) and found that these viruses accounted for 80% of the viral infections [147]. Another study tested nearly identical viruses, similar to our study, in nasal and pharyngeal swabs collected form patients with ARI (0 to 14 years) in Bavaria, Germany, and detected at least one virus in 77% of the [148]. In addition, at least one virus was detected in 79% of a pediatric group from Greece (< 17 years old) [149]. However, lower viral detections were observed in France (51%) and Belgium (62%) [116, 146], while higher detection rates were observed in children from the United Kingdom and Spain (92%) [150] compared to the inception cohort.

The most frequently detected virus in current study was HRV (22%), followed by RSV (17%). As in previous reports, HRV was the most commonly detected virus in children with ARI, with about 30% in Germany [151, 152] and 28% in France [146]. Conversely, RSV was the most frequent viral pathogen in Germany and other European countries, followed by HRV. The

incidence of RSV in the current study was lower than that reported from Germany (32%)[147] and the United Kingdom and Spain (53%) [150]. The lower incidence rate of RSV in this study might be caused by the enrollment of specific patients with ILI, which focused on fever and one respiratory symptom. Hombrouck et al., however, reported a comparable incidence of RSV (19%) in children less than five years old with ILI from Belgium, in which RSV was second to IV (20%) followed by HRV (17%) [116].

RSV is an important pathogen in infants ≤ two years old with ILI, particularly in the first year of life [153, 154]. In this study, most RSV-infected patients were infants and young children less than two years old, which accounted for 23.4% of all specimens investigated in this age group. The occurrence of RSV infection decreased with increasing age, with 13.6% for children aged 2 to 4 years old and 2.5% for children aged ≥ five years old. This trend was confirmed by long-term studies from Germany, including publications examining the impact of RSV on pediatric inpatients [145, 155, 156] and among all ages [153].

Interestingly, HBoV was detected in 16% of the children with ILI in this study. HBoV was most prevalent in children younger than two years of age. Of them, 31% (292/944) were positive for single infections. The role of HBoV in disease etiology is not fully understood. It has been hypothesized that HBoV may be located in the respiratory tract as a harmless bystander rather than a true pathogen [157], which is supported by the fact that HBoV is detectable in asymptomatic patients [158] and in healthy population [159]. Based on PCR assays, a comparable incidence of HBoV infection was identified in respiratory specimens derived from 254 children with ARI in Duesseldorf, Germany (19%), and 94 pediatric in-patients with severe LRTI aged less than 3 years in Aachen, Germany (13%) [160]. However, the incidence of HBoV in this current study was higher than that previously reported in children with ARI from France (4%) [146], but lower than that reported for British (20%) and Spanish children (24%) with ARI [150].

After HRV, RSV, and HBoV, IV (11%) and HAdV (9%) were observed as the fourth and fifth most frequently detected respiratory viruses, followed by HPIV (6%), HCoV (5%), and HMPV (4%) in our pediatric setting. Respiratory viruses HRV, IV, RSV, HAdV and HPIV usually rank among the top five of acute respiratory infections. Depending on the study design, patient's age, type of specimens and detection method, the order of these pathogens can vary, but incidences are in a comparable range across all the studies [147, 149, 151, 152, 156]. With respect to virological surveillance of ARI in children in Lower Saxony, Germany, there was

an even distribution of respiratory viruses in children as determined in a cohort study, except for IV, which was slightly increased (21% HRV/enterovirus, 18% IV, 17% RSV and 9% HAdV). Within the German National Outpatient Sentinel Surveillance for IV (https://influenza.rki.de/Default.aspx), IV (30%) was most frequently detected in pediatric and adult patients, followed by HRV (12%), RSV (9%), HAdV (4%), and HMPV (2%) [161]. It is worth noting that IV was detected on average in only 11% of the specimens, despite the fact that only children with ILI were enrolled in the inception cohort. A probable reason might be that the majority of the study cohort was aged < 2 years (57%), with a median age of 1.2 years. These children were predominantly infected with RSV, HAdV, HRV, HBoV, HPIV and HCoV (Chi-squared test, $p \leq 0.001$). For confirmation, children aged 2 to 5 years old (24%) and older than 5 years (19%) were most likely to be infected with IV ($p \leq 0.001$). Similarly, the German National Influenza-Surveillance observed that infants and young children (≤ 1 year) were most commonly infected with RSV and HRV, while within all other age groups, infection with IV was predominant [161]. Another German prospective study among children with either ILI or ARI reported an incidence rate of IV (11%) similar to that observed in our study [147]. Other pediatric studies from Germany (1.8%–8.6%) [145, 151, 162] or European countries (4.5%–16%)[132, 146] identified lower incidence rates compared to our cohort study. The incidence rate of IV in pediatric studies seems to be lower than in studies that include both children and adults [116, 149, 163].

Of all respiratory viruses investigated, HMPV showed the lowest incidence rate in the cohort study (average value of 4%). It should be noted that HMPV incidence rates varied from 2.1%–15.4% over the seven consecutive seasons analysed. Variation in low and high incidence rates has also been observed by a 10-year retrospective analysis of HMPV in children with ILI within the German national influenza surveillance system, in which incidences varied between 2%–17%) [164]. In addition, most pediatric studies from Germany (4%–6%) [152, 165] and other European countries (2.7%–3.5%)[146, 166], as well as national surveillance data among all ages of individuals in Europe (England and Wales: 2.2% [26], Germany: 3.0% [164], and Greece: 6% [167]), observed similar lower than average incidence rates for HMPV when compared to the top five ranking respiratory viruses.

HPIV and HCoV were analyzed mostly in children with ILI or ARI in Europe. Incidences between 2.5% and–7.6% for HPIV and between 2.5% and –5.8% for HCoV were observed [145,

[146, 150-152, 156, 166]. These findings have been confirmed by this study (HPIV, 6.3% and HCoV, 4.6%)

With the development of multiplex RT-PCR assays, the possibility of detecting a comprehensive set of respiratory pathogens suddenly increased. Today, multiplex assays are used increasingly in clinical diagnostics, enabling fast identification of not only a probable single pathogen, but also of co-infections. Comparable viral co-infection incidence rates of (13%–24%) have been observed among children with ILI or ARI by several studies in European countries [146, 149, 152]. A similar frequency of co-infections was observed in 19% (1135/6042) of all specimens investigated in the cohort study, in which 968 (23%) were double-, 143 (3%) were triple, 23 (0.6%) were quadruple infections, and one (0.02%) quintuple infection was detected. Most commonly, combinations of RSV-HBoV[17, 150], RSV-HRV [146, 148, 150], HBoV-HRV [146] and HAdV-RSV [146, 150] were detected as observed elsewhere. In addition, little attention has been given so far to the combination pattern of HAdV-HRV, HBoV-HAdV and HBoV-IV, but these patterns were identified in 141 cases, 132 cases and 104 cases, respectively, within this study [168]. HBoV are mostly regarded as co-infecting viral pathogens and seldom as an agent of single infection [150, 169]. The rate of co-infections with HBoV was high in the present study (69%) and it was in agreement with previous reports [17 150, 170, 171]. HAdV was also present mainly as a co-infecting pathogen. Bezerra et al. have reported a similarly high rate of HAdV co-infection (53%) as determined in a cohort study (56%) [171]. Currently, the mechanism of virus-virus co-infection remains unclear [168]. However, there are some plausible explanations. Differences in epidemiology and overlapping seasonal circulation of viruses have been suggested by Cebey-Lopez et al. to explain the variations between combinations of viral co-infections [150]. HBoV, HRV and HAdV circulated throughout the year; therefore, it is not unlikely that co-infections of these three pathogens occurred with RSV and IV. In addition, considering frequent and prolonged shedding of HBoV, HAdV and HRV [170, 172, 173], it is not surprising that these viruses were commonly detected in co-infection.

Generally, several surveillance systems and studies investigated the seasonal circulation of respiratory viruses. IV is likely to have an annual seasonal circulation with intensive circulation during the winter months in temperate regions [163]. However, a less defined seasonal pattern is observed in tropical areas with multiple peaks and a high background IV of activity year-round [174, 175], indicating that environmental factors may influence IV seasonal circulation [176]. In the present study, an IV wave was observed annually between December

and April. It peaked in January or February, as reported by the German national influenza-surveillance system of the AGI [177] and other European countries, such as the United Kingdom and Belgium [178, 179]. One potential explanation is that cold weather and low humidity may facilitate transmission and occurrence [176]. Another possible explanation is frequent close-contact transmission in crowded households when the temperature is low outdoors.

HMPV followed a regular circulating pattern between winter and early spring, which is in line with observations from Germany [164]and other countries located in temperate regions [180-182]. In Austria, HMPV showed a biennial rhythm for alternating epidemic early season (winter peaks during December to February) and late season (spring peaks during April and June), and early season onset was more likely associated with a high incidence of HMPV circulation and vice versa.[183] This observation was confirmed by findings from China, in which HMPV predominately circulated from November to February and April to June [184]. However, such biennial patterns of HMPV infection were not observed in the current study, possibly due mainly to the fact that most specimens were collected in the cold season (Figure 4). In the present study, a significantly intensive circulation of HMPV was seen only in the years 2009, 2014 and 2015, as reported by a population-based study [164]. Although the seasonal circulation of HMPV infection is fully understood, it is likely to be influenced by climate and timing of seasonal epidemics and may vary by location [184, 185].

In this study, RSV infections peaked in the winter months as observed by other regions with a temperate climate [146, 163, 186]. Activity of RSV circulation varied significantly over seven consecutive seasons, and a regular biennial pattern of alternating early and late season occurrence was observed. This finding is supported by previous studies from Germany and other European countries including Switzerland, Sweden and Finland [153, 187-189]. Additionally, this study also confirmed the findings that early seasonal onset is correlated with intensive seasons and *vice sera*, as previously reported by European countries, including Germany [155, 187-191].

HRV, HPIV, HAdV, HBoV and HCoV circulated throughout the year. HRV had a higher incidence in the autumn months and no clear seasonality was observed for HAdV, HBoV and HCoV. Similar circulation patterns have also been determined by the Respiratory Viruses Network (RespVir) [192]. Previous studies also confirmed these findings [145, 146, 163, 193-195]. Although HPIV circulated year-round [145, 146, 163], subtle differences in regarding peak circulation among reports/studies were observed. In contrast to the slightly circulating peak

during the winter months [163], the current study and another European study [146] observed a slightly higher occurrence of HPIV during summer-autumn months (less or no circulation during the winter months). Differences among the participants studied may explain the subtle variation of circulating peak peaks. All ages of populations were included in a study by Ramaekers et al. [163], whereas only children with ARI or ILI were enrolled in the current study and the study by Fillatre et al. [146]. As shown above, respiratory viruses, including HPIV tend to occur in young children.

9.2 Respiratory viral infections in children cannot be distinguished by a single clinical feature

Parts of this discussion section have been accepted for publication [53].

With the introduction of novel vaccines and antiviral medications, it has become important to quickly and accurately differentiate respiratory viral infections in the early stage of diseases, particularly in children accurately and in a timely manner. To our knowledge, there have been two systematic literature reviews and meta-analyses dedicated to the relationship between respiratory viral pathogens and specific clinical signs and symptoms. One meta-analysis (Ebell et al. [196]) investigated the symptoms for IV in children and adults, whereas the second study (Thornton et al. [197]) focused on children and included the same eight respiratory viruses as in this cohort study, plus three common bacterial pathogens (*Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*). In comparison to both meta-analyses, this literature review included more recent publications (Ebell et al., up to 2001 [196]; Thornton et al., up to 2014 [197]) [53]. Moreover, the current meta-analysis included a larger sample size (49858 versus 6790 [196] and 15069 [197], respectively) and more countries (24 versus 4 [196] and 20 [197], respectively) [53]. In this study, meta-analyses were performed for the first time within a literature and cohort dataset and directly compared to each other based on the same statistical algorithms.

No individual clinical feature alone was unique to a specific type of respiratory viral infection, with the exception of headache/IV and diarrhea/HAdV. For instance, the present study identified fever to be most likely associated with IV, as previously stated in a meta-analysis [196]. In addition to IV, fever is also likely to be associated with HAdV, which is also supported

by Giamberardin et al. [6]. The present study further reported that HAdV infection was clinically similar to IV, both of which shared symptoms, such as rhinitis and sore throat, which were not reported in the work by Giamberardin et al. [6]. In addition, to confirm the finding that wheezing/LRTI was strongly correlated with RSV [197], the present study also revealed that wheezing was also commonly linked to HMPV and HBoV. The lack of relationship with other viral pathogens in a previous meta-analysis [197] might be explained by its limitation to children with acute cough. Similarly, we not only confirmed the finding that infections with RSV or HMPV are clinically indistinguishable [198-204], but also found that clinically indistinguishable viral infections with RSV or HMPV should include HBoV, which has not been observed in a previous 2-year pediatric study [199].

The comprehensive analysis showed that systemic symptoms, such as headache and diarrhea, were individually linked to one viral pathogen. Headache was solely associated with IV. Similarly, in Australian children suffering from ILI, headache and myalgia were more common in patients positive for IV A/H1N1/pdm09 than in patients positive for other pathogens [103]. However, the authors did not determine the specific type of respiratory (viral) infections among the non-IV groups. Furthermore, in the inception cohort, the number of patients enrolled was large (N = 6042) and even larger in the literature review dataset focusing on IV (N = 24661). On the basis of both datasets, headache was significantly associated with IV infection, but negatively correlated to the other seven types of ARI except for HRV. In the literature dataset, headache showed a positive association with HRV, but it was not statistically significant. Indeed, HRV was significantly negatively associated with headache in the cohort dataset. Compared to the inception cohort (N = 6042), the literature dataset was less representative for HRV (N = 2653). Thus, the association between IV infection and headache seems plausible. However, it must be pointed out that headache as a predictor of IV infection is a challenge in most young preverbal children, who cannot reliably express subjective symptoms, such as headache.

Moreover, diarrhea was significantly associated with HAdV in both datasets. HAdV infections in children with ARI were typically reported to include fever, cough, rhinorrhea, sputum, rales and gastrointestinal symptoms, including diarrhea and swelling of tonsils [205-208]. With respect to fever and diarrhea, none of the other common presentations were identified to significantly correlate with HAdV infection in either dataset, although rhinitis (or rhinorrhea) and pharyngitis (sore/red throat) were significantly associated with HAdV infection in the

inception cohort dataset. However, HAdV types 40 and 41 are frequently reported to be associated with gastroenteritis including diarrhea [209-211]. Since no type differentiation was performed in the present study, this association cannot be ruled out. Further, HAdV persisted in specimens over a long period of time, even when the patients were no longer ill [212], and HAdV may not be the primary pathogen causing the respiratory infection. In support of this opinion, the high co-infection rate (56%) of HAdV-infected patients was detected in this cohort study, suggesting that the role of HAdV in respiratory illness may be overestimated.

Compared to the meta-analysis of the literature dataset, the cohort dataset also established new significant associations between individual clinical features and viral infections. Usually, "desired" (positive) results are more likely to be published than "undesired" (negative) ones. Negative associations therefore might be missing in meta-analyses of the published literature. A negative association of fever with RSV was identified in the inception cohort dataset, but not in the pooled literature review dataset. Furthermore, this negative association between fever and RSV has been supported by previous studies [112, 114, 123]. Conversely, several studies reported that RSV-infected patients more frequently presented with fever compared to those without RSV infection [98, 110, 142]. These contradictory findings may be explained by the fact that fever is intermittent in RSV-infected cases, according to Nelson's Textbook of Pediatrics [213].

The individual studies in the literature review dataset showed high levels of heterogeneity, especially with regard to inclusion criteria and/or cut-off criteria for specific symptoms such as fever and hypoxia [53]: For example, seven studies used a definition of blood oxygen saturation level of < 90% to define hypoxia, while two studies use a higher threshold-blood oxygen saturation at either < 92% or < 95% [102, 132]. Similarly, 11 studies defined fever as a body temperature ≥ 38°C, while six studies used thresholds of 37.5°C [100, 108, 110, 113, 124, 137] and one study used a threshold of 38.2°C [105]. However, 22 studies did not address cut-off limits for fever definition. In addition, the literature review methodology was limited by the inconsistency of methods used to detect respiratory viral infections and describe the clinical presentation [53]. Each published study used slightly different laboratory and clinical data collection methods, including phone/interviews[6, 97, 102, 110, 112], questionnaires [99, 100, 109, 113, 117, 121, 124, 130, 140]and surveys [142]. By contrast, the design of the inception cohort limited the risk of bias and heterogeneity through standardized clinical assessments in a pre-defined group of patients followed by independent laboratory and data analysis [53]. In inception cohorts, the

same data are collected from all patients and the same definitions/cut-off criteria for symptoms such as fever and hypoxia are used consistently. Standardized clinical and laboratory data collection in the QM program included pre-defined positive and negative findings, yielding a complete dataset for the analysis of positive and negative associations [53]. Subjective symptoms, such as headache and myalgia, may be underreported in infants and young children compared to older children [53]. Headache and myalgia can only be elicited by age-related examination techniques. To avert observer bias in the cohort dataset, a trained QM team elicited these symptoms accurately in all patients, regardless of age [53]. Further, narrower CIs were observed in the prospective inception cohort dataset than in the literature review dataset. The sample size of each enrolled literature study varied considerably, with the sample size ranging from 48 [111] to 14479. [118] This may explain the estimated wide CIs of meta-analysis for several studies with a small sample size, indicating a less precise estimation. The inception cohort enrolled 6042 children patients, and clinical features were collected for each patient. Thus, precise CIs were obtained. This indicated that a prospective/inception cohort dataset might give a more precisely diagnostic effect.

The (meta-) analyses of both datasets hardly revealed any individual clinical features to be able to distinguish between specific types of respiratory viral infections. Moreover, several symptoms and/or signs were found to be associated with two or more respiratory viruses. Clinicians should bear in mind that an individual symptom alone cannot rule-in or rule-out a specific type of respiratory viral infection.

9.3 Novel decision tree models for predicting respiratory viral infections in children

A variety of respiratory viral pathogens are major causes of ARI in young children (see 4.1). Currently, specific antivirals, e.g., NAIs, are only available for IV infection. Thus, it is crucial to differentiate between IV and other respiratory virus infections in children. However, current case definitions or decision tree models focus mostly on IV rather than other respiratory viruses [47-49, 79, 83, 214]. Furthermore, almost all of these decision tree models used CART algorithm and were conducted in adults, in which only symptoms were included.

The present study addressed some proposed new topics in decision tree modelling [83]. In addition to symptoms (model 1), decision tree models were also calculated for clinical variables (model 2) and prevalence data (model 3) using the CIDT algorithm. Moreover, novel CIDT models were calculated for not only predicting IV infections, but also RSV, HMPV, HRV, HAdV, HCoV, HPIV and HBoV-1 infections.

All three CIDT models calculated showed the highest sensitivity (IV, 60.8%–71.9%; RSV, 66.5%–80.5%) and specificity (IV, 64%–89%; RSV, 58.8%–83%) for predicting IV and RSV infections. Lower sensitivity/specificity was determined for HRV (models 1-3 sensitivity: 25%–36.3%), HBoV-1 (CIDT models 1–2 specificity: 10.9%; CIDT model 3 sensitivity: specificity 53.9%) and HAdV (CIDT models 1–2 sensitivity: 42.2% and specificity: 51.9%; CIDT model 3 specificity: 35.6%). The lower sensitivities/specificities of the CIDT models for HRV, HBoV-1 and HAdV infections may be explained by three reasons: (1) a high co-infection rate, (2) no clear seasonality, and (3) non-specific defined symptoms. It is worth noting that CIDT predictive models of HCoV and HPIV did not work for models 1 and 2 (no outputs of variable), and the poor sensitivity/specificity of model 3 (HCoV sensitivity: 25% and HPIV specificity: 0%-29.5%) was revealed. The possible reason for this was the small number of HCoV- and HPIV-infected patients (N = 275 and 378 patients, respectively) and the large number of co-infections for HCoV (62%, 169/275). The sample number of HMPV-infected patients was small (N= 275), but the CIDT models showed moderate sensitivity (57.5%-68.5%) and specificity (60.5%-76.5%). CIDT modelling for predicting HMPV appears to be a promising approach. Consequently, these analyses should be repeated in an enlarged setting with HMPV-positive children.

9.3.1 CIDT models for predicting IV

We developed three CIDT models for predicting IV infection. Model 1 included LRTI, cough, fever, headache, seizure and need for hospitalization as variables; model 2 included LRTI, cough, fever, need for hospitalization, dyspnea, and children younger than 2 years as variables; and model 3 included fever, children younger than 2 years and seasonality as variables.

IV model 2 yielded a higher AUC and specificity than IV model 1, included the age threshold for children as a divisor, and classified more patients into a low risk group which required neither testing nor treatment (1 versus 5 patients). Alternatively, model 1 classified relatively

more patients into a high-risk group, which required empirical treatment (256 versus 226 patients) with a higher sensitivity than IV model 2. Compared to IV CIDT models 1 and 2, IV CIDT model 3 yielded a higher AUC and specificity (sensitivity was comparable with IV CIDT model 2, but higher than CIDT model 1) and classified more patients into a low-risk group (35 patients) or high-risk group (272 patients). Unlike IV CIDT models 1 and 2, IV CIDT model 3 utilized the distribution of IV circulation, which was in agreement with the knowledge that diagnosis of IV should be linked to prevalence data of circulating IV infection in the local community [83]. In general, IV CIDT model 3 provided the most clinically promising rules to predict the likelihood of IV infection. All of these predictive models are rational options depending upon the preferences of the patient candidates and clinicians. These predictive CIDT models are only generated by statistical methodology; Thus, whether it works well for clinicians still needs to be validated by larger and multi-centre prospective cohort studies and laboratory PCR assays.

Few CART models have been reported to evaluate the predictive capacity of IV diagnosis in populations presenting with ARI or ILI [79, 83, 214]. Compared to CART models for predicting low- and high-risk IV by Afonso et al. [83], IV CIDT model 3 in this study yielded a higher AUC value (0.75–0.76 versus 0.86), but included more variables (fever ≥ 37.3°C, and chills/sweating versus fever ≥ 38°C, children younger than 2 years and seasonality). Chills were recorded as baseline, but were not included as a parameter in the CIDT models in the present study. Zimmerman et al. used the CART algorithm to develop models for predicting IV infection and included fever, cough and fatigue in children younger than 5 years with ARI (sensitivity: 84% and specificity: 48%) and fever and cough in all patients aged ≥ 5 years (sensitivity: 86% and specificity: 47%) [79]. Similarly, using the CART algorithm, Evers et al. included cough, IV incidence, IV contact and immunization status and produced a sensitivity of 84% and a specificity of 46% in children younger than 2 years with ILI. They also included myalgia, diarrhea, IV incidence (namely prevalence data) and immunization status, yielding a sensitivity of 89% and specificity of 33% in children aged 2–18 years with ILI [214]. In comparison, we reported IV CIDT model 3 using fever, children younger than 2 years and seasonality (prevalence data) and yielded a sensitivity of 70% and specificity of 89%, significantly improving the specificity for predicting IV infection. Thus, this IV CIDT model 3 is very effective at identifying a group who does not require either diagnostic tests or antiviral treatment. We did not collect information regarding IV contact, and the immunization status against influenza was low (8.2%) in the inception cohort. Therefore,

these parameters were not initially included in CIDT models as recursive partitioning parameters. In future work, we or other researchers will perform the CART algorithm to predict IV in this same cohort dataset, and better interpret the differences of output between CIDT and CART.

These CIDT models have several strengths. First, these IV CIDT models particularly CIDT model 3, significantly improved the specificity of predicting IV infection using CART models [79, 83, 214]. Second, these three IV CIDT models collectively confirmed that fever was a crucial symptom for ILI case definition [47-49] and that it yielded a higher sensitivity than the current international ILI case definition recommended by the WHO, CDC and ECDC [36, 50]. The variables 'moderate/high seasonality' and 'fever' as defined by the decision tree analysis (IV CIDT model 3) in this study reflected symptoms included in the ILI case definition by the CDC, which has been recently evaluated to be most sensitive for the detection of IV infection [36]. Furthermore, risk stratification based on the decision tree algorithm for predicting the likelihood of IV infection provides several benefits in decision-making, including antiviral treatment and testing. A low-risk group, in which neither testing nor antiviral treatment is recommended, is likely to economize medical resources and potentially minimize inappropriate antiviral prescription. In comparison, the high-risk group, in which empirical treatment is suggested, can aid in developing a guideline for antiviral prescription specifically against IV, such as NAIs in health care centres, particularly in primary care settings [215, 216].

Several limitations should be acknowledged when interpreting our interesting findings. First, the thresholds for testing and treatment used in the CIDT models were referred to in reports by two pediatric studies [143, 144]. These testing and treatment thresholds need to be validated in further work. Second, although the sensitivity of IV CIDT models is higher than international ILI case definitions, it is under 72%. Thus, for those "positive" IV patients suggested by these IV CIDT models, the decision to order either diagnostic tests or antiviral treatment should be based on clinical judgement. In addition, decision tree modelling is a statistical approach, which must be proven by daily routine in clinics. Therefore, next to clinical judgement, laboratory testing in high-risk patients is needed to evaluate this algorithm.

9.3.2 CIDT models for predicting RSV

Compared to the RSV CIDT model 1 (variables: LRTI, cough, headache, seizure and rhinitis) and RSV CIDT model 2 (variables: LRTI, cough, headache, seizure and children under 2

years of age), RSV CIDT model 3 (variables: LRTI, cough, children under 2 years of age and seasonality) had the best overall predictive value with a relatively high specificity (83%) and sensitivity (70%). Surprisingly, there is no decision tree model currently available for predicting the RSV CIDT algorithm. Additional interactions of contributing variables were potentially used in the RSV CIDT algorithm relative to logistic regression methodology (LRM). For instance, Park et al. have showed that LRTI but not cough is an independent predictor of RSV infection [217]. Conversely, Loscertables et al. revealed that cough rather than LRTI is an independent predictor for RSV infection [218]. Both studies used traditional LRM. The present RSV CIDT model 3 not only simultaneously determined independent predictors for RSV, such as LRTI and cough [218], but also included children under 2 years of age and seasonality. It is worth noting that the CIDT algorithm as used in the present study usually investigates the interaction of several shared variables instead of determining individual ones. In contrast, independent predictors determined by LRM, i.e., direct comparison between these two methodologies, should be performed carefully.

Because of higher specificity, RSV CIDT model 3 is likely to identify “true negative” RSV infection (needing neither testing nor treatment). Currently, licensed antiviral medications are available for influenza but not for other respiratory viral infections, including RSV. Thus, children identified as being at the highest risk of RSV infection do not also need to be tested for IV and/or administered with antivirals against influenza. However, caution should be exercised by clinicians when identifying “true positive” RSV infection (e,g., high-risk RSV infection) using RSV CIDT model 3 due to a sensitivity of 70%. Although RSV CIDT model 2 was better at determining high-risk patients with RSV infection than RSV CIDT model 3 (sensitivity: 81% versus 70%), its specificity is considerably lower (59% versus 83%). Collectively, RSV CIDT model 3 showed the most promise for clinicians in routine care.

10 Conclusion

The present study has demonstrated that many clinical features were shared by various types of respiratory viral infections, i.e., individual clinical features alone provide limited value in predicting any specific type of respiratory viral infection. Development of novel clinical decision rules using CIDT algorithms for the prediction of IV or RSV infection yielded a sensitivity of 70% and specificity of more than 83%. Thus, these models would be suitable to identify children at high risk of suffering from IV and RSV infections. With regard to IV, laboratory testing or treatment based on clinical judgement (including antivirals) should be recommended for patients at highest risk, while laboratory testing for IV should be considered based on clinical judgement for patients at moderate risk. Low-risk patients need neither IV testing nor treatment with antivirals against IV. Children identified as the highest risk for RSV infection also do not need to be tested for IV and/or be treated with antivirals against IV. Interestingly, the variables 'moderate/high seasonality' and 'fever' as defined by decision tree analysis in the present study reflect symptoms included in the ILI case definition by the CDC, which has been recently evaluated to be most sensitive for the detection of IV [36].

With regard to the comparative meta-analysis of literature review and cohort data, it has been shown that the QM inception cohort provided reliable clinical data, which is necessary for setting up such meta-analysis or decision tree models. Although the inception cohort provided a large sample size with 6042 PCR-analyzed specimens, the number of samples for each of the seven consecutive seasons was comparatively small. Thus, for the respiratory pathogen HMPV, further inception cohorts with a larger annual sample size would not only enhance the respective positivity rate but also the likelihood for the prediction of HMPV by decision tree analysis.

Even though significant associations between clinical features and types of viral infections have been identified, clinicians should be aware that individual clinical features cannot rule-in or rule-out any specific viral infection [53]. Moreover, decision tree modelling as a statistical approach needs to be proven in the daily routine of clinics. Diagnostic testing for respiratory viruses will remain the cornerstone of accurate diagnoses [53]. Testing should be recommended to evaluate the decision tree algorithm and be encouraged to prevent unnecessary prescriptions of antivirals in "similar-looking" non-IV cases, where NAIs would be ineffective [53, 219, 220].

11 Bibliography

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12 Affidavit

I, **Xiaolin Ma**, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic „**Key clinical features and epidemiological patterns associated with influenza and other respiratory virus infections in children – a prospective cohort study**“. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My interest in any publications to this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____ _____

Date Signature

13 Declaration of individual contribution to publications

1. **Xiaolin Ma**, Tim Conrad, Maren Alchikh, Janine Reiche, Brunhilde Schweiger, Barbara Rath. Can we distinguish respiratory viral infections based on clinical features? A prospective pediatric cohort compared to a systematic literature review. **Reviews in Medical Virology (**Accepted 15 June, 2018; in press) (**IF: 5.034**)

Xiaolin Ma performed laboratory experiments for the investigation of human coronavirus (HCoV-NL63, 229E, OC43 and HKU1), human parainfluenza virus (HPIV 1-4) and human bocavirus 1 (HBoV-1) (nucleic acid extraction, reverse transcription of viral RNA), and real-time RT-PCR analyses. He analyzed respective results gained from his experiments. Further, Xiaolin Ma conducted the systematic literature research and interpreted the literature review data. Both virological and clinical data obtained within the inception cohort and the literature review, Xiaolin Ma prepared for the statistical analyses, which was performed by Tim Conrad. Xiaolin Ma wrote the initial draft of the manuscript and revised final draft. He has seen and approved the final version of the manuscript.

2. Maren Alchikh, Tim Conrad, C Hoppe, **Xiaolin Ma**, Eeva Broberg, Pasi Penttinen, Janine Reiche, Barbara Biere, Brunhilde Schweiger, Barbara Rath. Are we missing respiratory viral infections in infants and children? Comparison of a hospital-based quality management system with standard of care. **Clinical Microbiology and Infection**. 2018 Jun 12, doi: 10.1016/j.cmi.2018.05.023. [Epub ahead of print], pii: S1198-743X(18)30458-0. (IF: 5.394)

Xiaolin Ma contributed to analyze parts of respiratory specimens (6.3%, 379/6042) for human adenovirus (HAdV), human rhinovirus (HRV) and human metapneumovirus (HMPV). Xiaolin Ma contributed significantly to the writing of the manuscript. He has seen and approved the final version of the manuscript.

3. Barbara Rath, Tim Conrad, Puja Myles, Maren Alchikh, **Xiaolin Ma**, Christian Hoppe, Franziska Tief, Xi Chen, Patrick Obermeier, Bron Kisler, Brunhilde Schweiger. Influenza and other respiratory viruses: standardizing disease severity in surveillance and clinical trials. Expert Review of Anti-infective Therapy. 2017; 15(6):565-568. (IF: 3.141)

Xiaolin Ma conducted the research of the systematic literature review and interpreted the systematic literature review. He has seen and approved the final version of the manuscript.

Signature, date and stamp of the supervising university teacher and second supervisor

Prof. Dr. Jörg Hofmann	Dr. Brunhilde Schweiger

Signature of the doctoral student

Xiaolin Ma

14 Curriculum Vitae

For reasons of data protection, my CV will not be published in the electronic version of my work.

15 List of publications

1. **Ma X**, Conrad T, Alchikh M, Reiche J, Schweiger B, Rath B. Can we distinguish respiratory viral infections based on clinical features? A prospective pediatric cohort compared to a systematic literature review. Reviews in Medical Virology 2018; 28(5):e1997 (**IF: 5.034**)

2. Alchikh M, Conrad T, Hoppe C, **Ma X**, Broberg E, Penttinen P, Reiche J, Biere B, Schweiger B, Rath B. Are we missing respiratory viral infections in infants and children? Comparison of a hospital-based quality management system with standard of care. Clin Microbiol Infect 2019; 25(3): 380.e9-380.e16
(**IF: 5.394**)

3. Rath B, Conrad T, Myles P, Alchikh M, **Ma X**, Hoppe C, Tief F, Chen X, Obermeier P, Kisler B, Schweiger B. Influenza and other respiratory viruses: standardizing disease severity in surveillance and clinical trials. Expert Rev Anti Infect Ther 2017; 15: 545-568. (IF: 3.141)

Before doctorate

4. **Ma X**, Wu F, Xin L, Su G, He F, Yang Y, Sun J, Liu Z. Differential plasma microRNAs expression in juvenile idiopathic arthritis. Mod Rheumatol. 2016;26(2):224-32. (**IF: 1.955**)

5. **Ma X**, Xin L, Sun J, Liu Z. Antinuclear antibody-positive cohort constitutes homogeneous entity in juvenile idiopathic arthritis. Mod Rheumatol. 2016;26(1):75-9. (**IF: 1.955**)

6. Sun J, Feng M, Wu F, **Ma X**, Lu J, Kang M, Liu Z. Plasma miR-26a as a Diagnostic Biomarker Regulates Cytokine Expression in Systemic Juvenile Idiopathic Arthritis. J Rheumatol. 2016;43(8):1607-14. (**IF: 3.470**)

7. Xin L, **Ma X**, Xiao Z, Yao H, Liu Z. Coxsackievirus B3 induces autophagy in HeLa cells via the AMPK/MEK/ERK and Ras/Raf/MEK/ERK signaling pathways. Infect Genet Evol, 2015,36:46-54. (**IF: 2.545**)

8. He F, Yao H, Wang J, Xiao Z, Xin L, Liu Z, **Ma X**, Sun J, Jin Q， Liu Z. Coxsackievirus B3 engineered to contain microRNA targets for muscle-specific microRNAs displays attenuated cardiotropic virulence in mice. J Virol, 2015, 89(2):908-916. (**IF: 4.368**)

9. Xin L, Xiao Z, **Ma X**, He F, Yao H, Liu Z. Coxsackievirus B3 induces crosstalk between autophagy and apoptosis to benefit its release after replicating in autophagosomes through a mechanism involving caspase cleavage of autophagy-related proteins. Infect Genet Evol, 2014, 26: 95-102. (**IF: 2.545**)

10. **Ma X**, Wu F, Su G, He F, Yang Y, Liu Z. The preliminary study of the relationship between special microRNAs in plasma and juvenile idiopathic arthritis. Chin J Appl Clin Pediatr, November 2013;28(21): 1614-1618.
11. Ma X, Wu F, Liu Z. The advanced study of microRNAs in rheumatoid arthritis. Chin J Rheumatol,2013;7(17):75-77.

12. Yang Y, Wu F, Kang M, **Ma X**, Yuan X. MRI manifestations of juvenile idiopathic arthritis involving hip joint and clinical relevance. Chin J Interv Imaging Ther,2013;10(3):147-150.

16 Acknowledgements

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Thanks a million to **Dr. Janine Reiche**, who has been supervising, caring, supporting and encouraging me throughout my doctoral project to the completion of dissertation writing. Thank you for sharing valuable time and research experience on our weekly meeting. Thanks a bunch to **Dr. Ralf Dürrwald, Prof. Michael Hummel** and **Dr. Sonja Gieseking** for kind help and thoughtful advices in the process of thesis.

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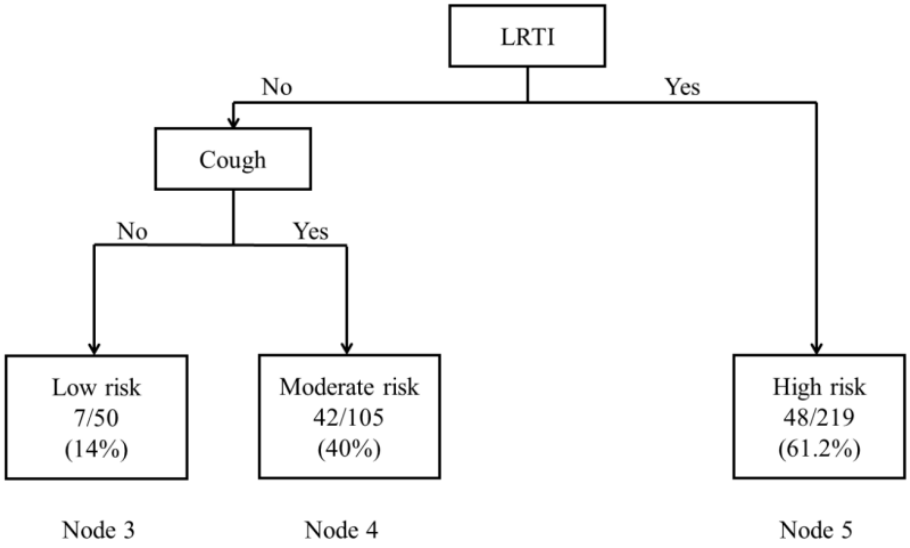
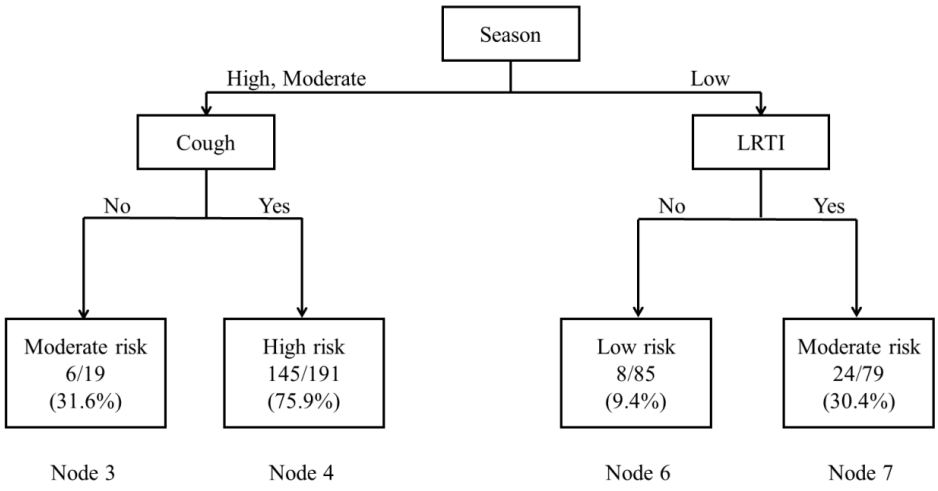
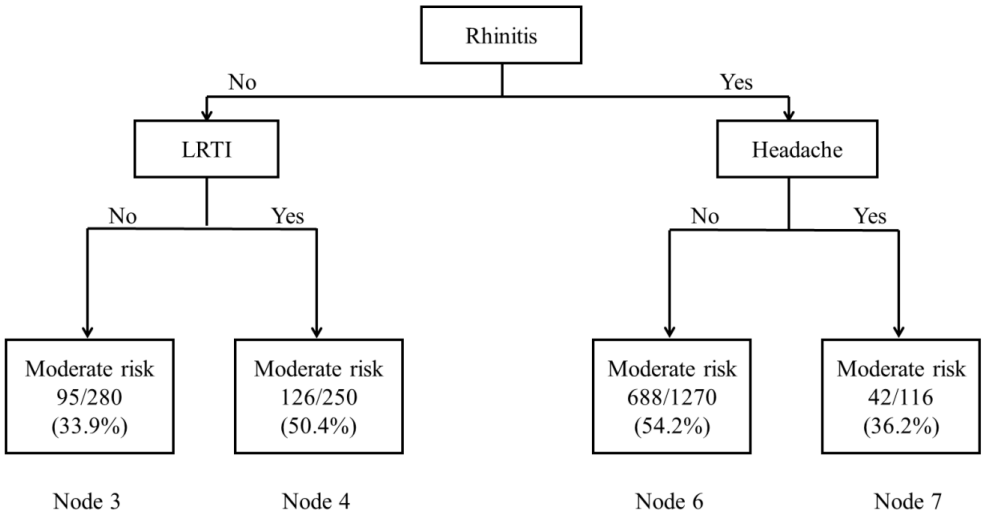
17 Appendix



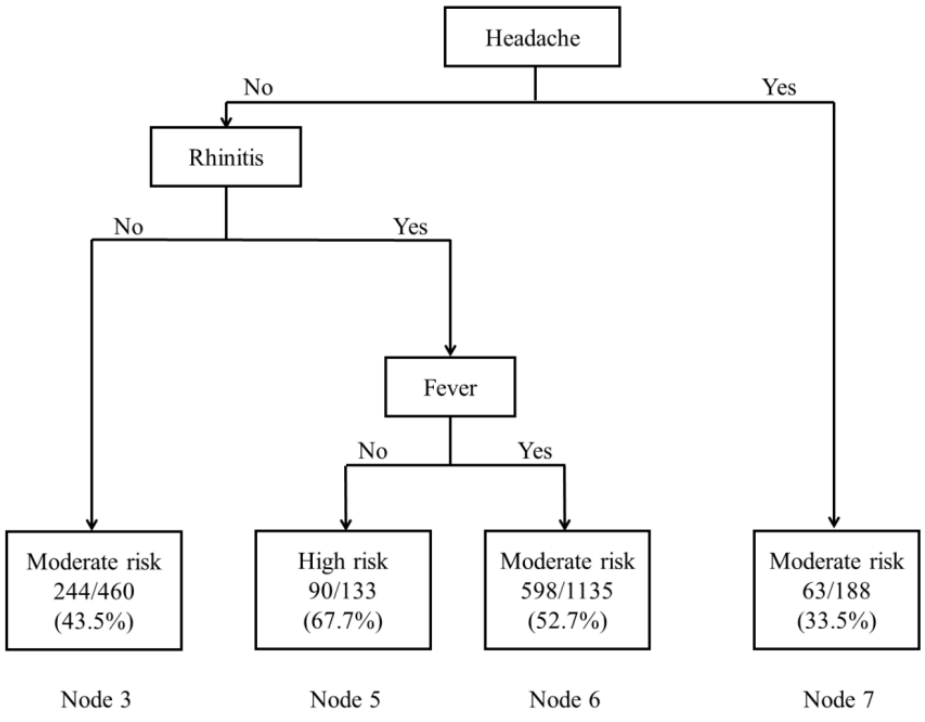
Appendix 1: A conditional inference decision tree (CIDT) model for predicting HMPV infection based on clinical features (HMPV CIDT model 1)



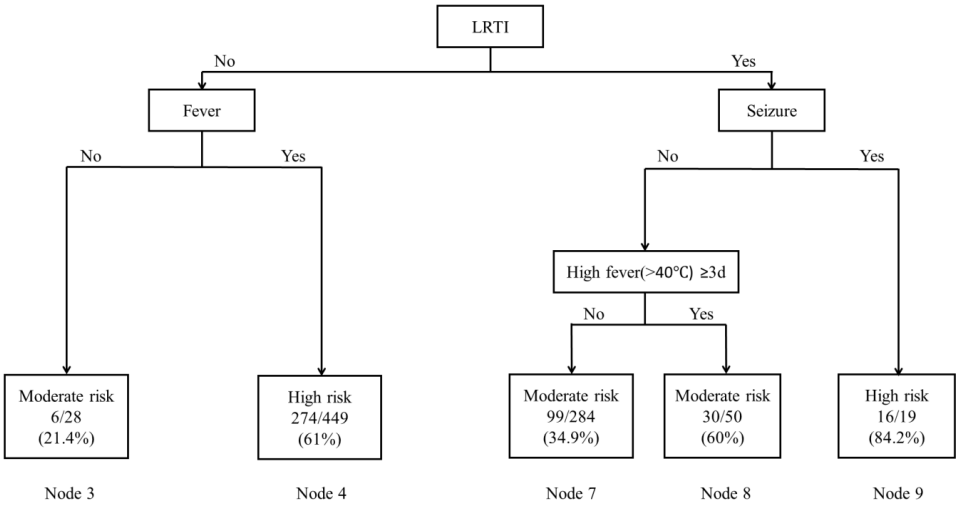
Appendix 2: A conditional inference decision tree (CIDT) model for predicting HMPV infection based on clinical features, risk factors and seasonal pattern (HMPV CIDT model 3)



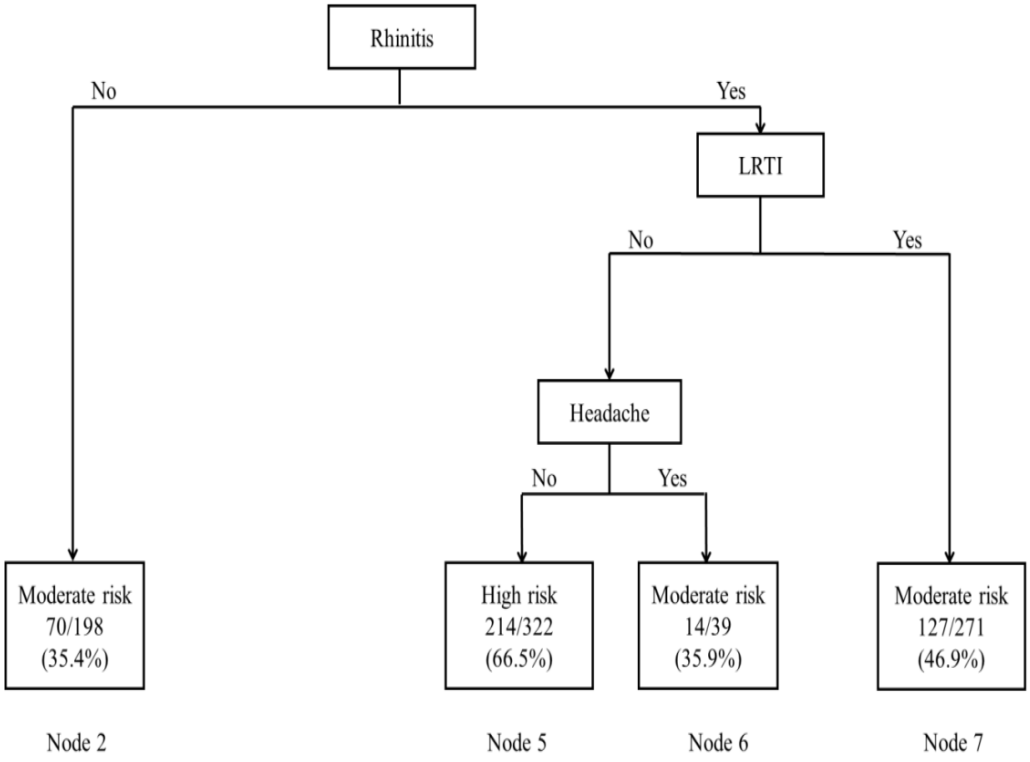
Appendix 3: A conditional inference decision tree (CIDT) model for predicting HRV infection based on clinical features (HRV CIDT model 1)



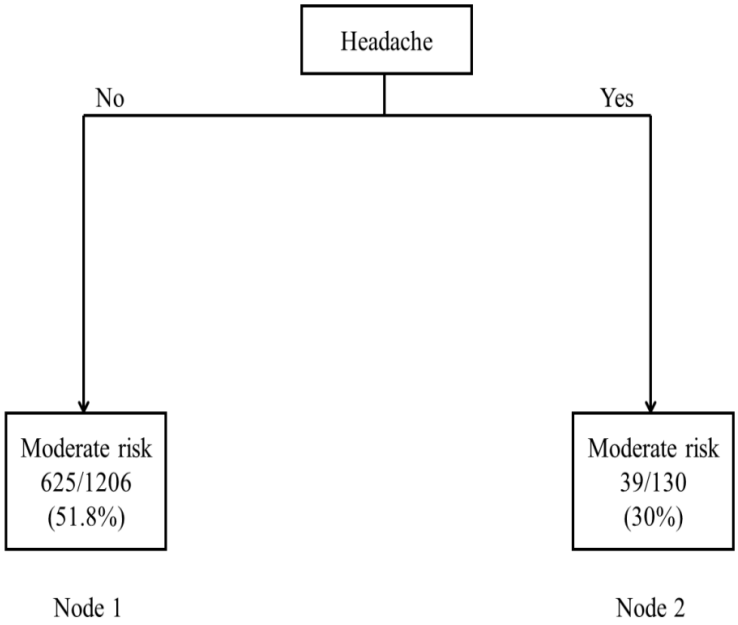
Appendix 4: A conditional inference decision tree (CIDT) model for predicting HRV infection based on clinical features, risk factors and seasonal pattern (HRV CIDT model 3)



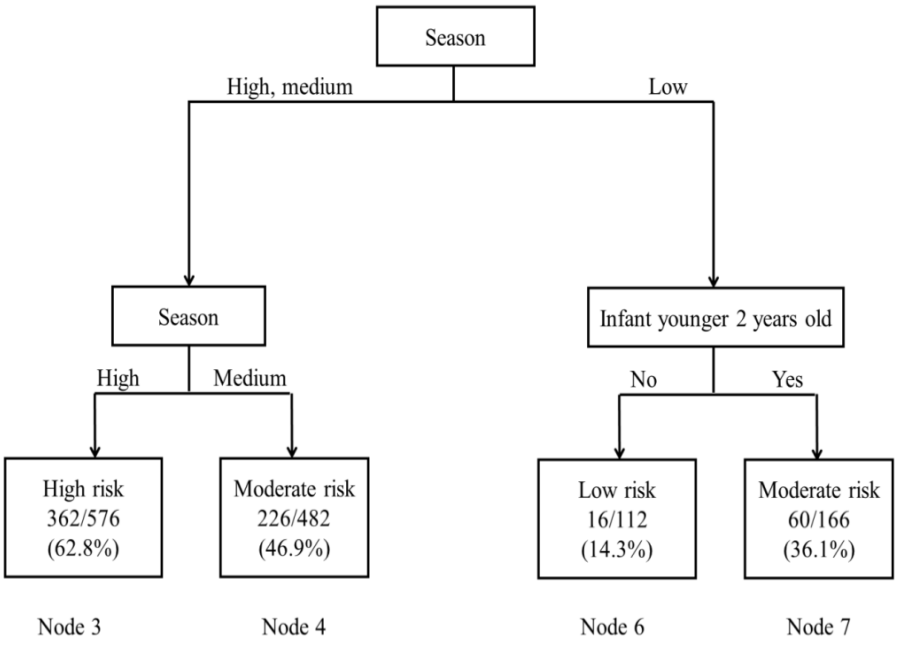
Appendix 5: A conditional inference decision tree (CIDT) model for predicting HAdV infection based on clinical features (HAdV CIDT model 1)



Appendix 6: A conditional inference decision tree (CIDT) model for predicting HAdV infection based on clinical features, risk factors and seasonal pattern (HAdV CIDT model 3)

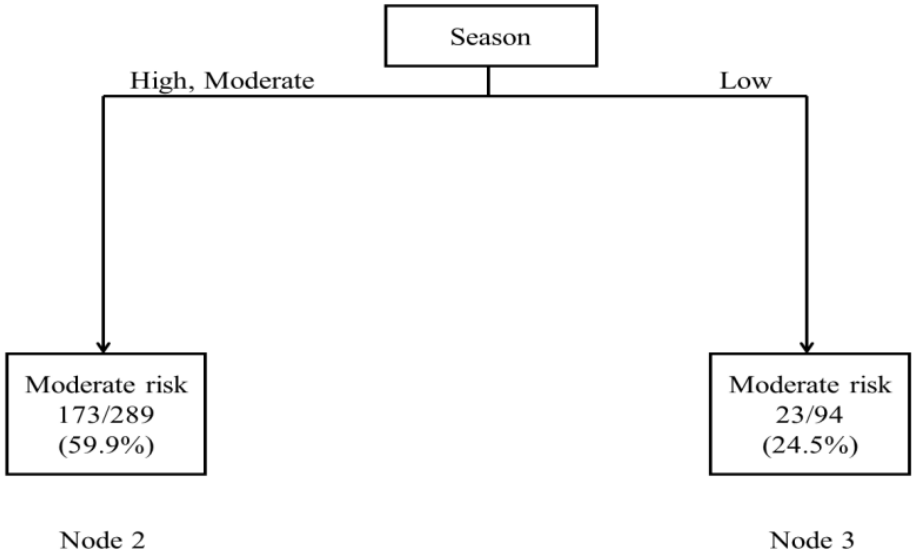


Appendix 7: A conditional inference decision tree (CIDT) model for predicting HBoV-1 infection based on clinical features only (HBoV-1 CIDT model 1)

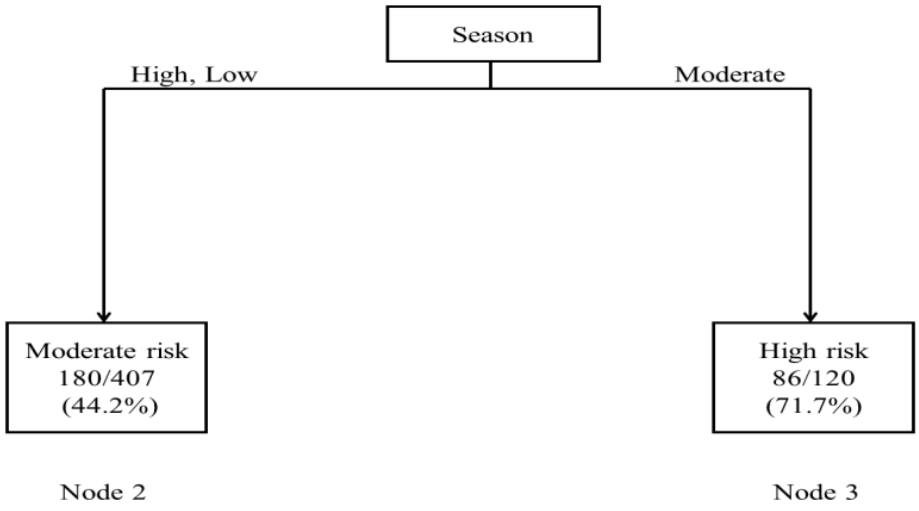


Appendix 8: A conditional inference decision tree (CIDT) model for predicting HBoV-1 based on clinical features, risk factors and seasonal pattern (HBoV-1 CIDT model 3)

a



b



Appendix 9: Conditional inference decision tree (CIDT) model for predicting (a) HCoV infection (HCoV CIDT model 3) and (b) HPIV infection (HPIV CIDT model 3) based on clinical features, risk factors and seasonal pattern