UNIVERSITY OF LIVERPOOL

Clinical and Pathophysiological Assessment of Wheezing in Children

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master of Philosophy by

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Abstract

Acute wheeze is common in preschool children. Historically, preschool wheeze has been poorly defined, and more focused research is needed. This work aims to enhance understanding of assessment and pathophysiology of preschool wheeze.

Preschool wheeze is highly variable in severity and the acute assessment is inconsistent. Severity scores allow standardised assessment. This can guide treatment and identify changes over time. A systematic review of the severity scores published for use in acute preschool wheezing illness was undertaken. The selection criteria and methods were pre-defined (PROSPERO ID CRD42020212507). This review found that 89 severity scores have been published for use in preschool wheeze. These scores included 24 domains, with 109 items. Auscultation and retractions were common (n= 85 scores). Many scores were setting-specific: 42 for use in ED/primary care and 36 for inpatients. No score was fully validated according to pre-set criteria. Only 37 scores had some validity data and 19 were unsuitable for young children. A validated severity score specific to preschool wheeze, and appropriate to the setting, should be used routinely, to improve research utility and clinical outcomes.

Wheeze exacerbations in preschool children are often viral induced, commonly by rhinovirus (HRV). The immunology is incompletely understood. A bronchial epithelium cell line, BEAS-2B, was cultured and infected with HRV-A and C. The expression of HRV receptors (ICAM-1 and CDHR3) and IL-6 mRNA were measured over 24 hours, using real-time quantitative PCR. All markers had an increased mean expression over 24 hours following infection, although none reached statistical significance. Further research is needed to fully characterise these relationships. Increasing evidence suggests ICAM-1 and CDHR3 as therapeutic targets for HRV infection.

A medical record analysis of admissions for viral-induced wheeze and asthma, between September 2015 and August 2020, to Alder Hey Children's Hospital was undertaken. Data included demographics, indicators of severity and social characteristics (deprivation and NO_2 exposure). There were 4263 admissions, with more males (64.3%) and 2-6 year olds (73.5%). There were temporal patterns in admissions, peaking in Autumn and Winter. The median length of stay (LOS) was one day. 29.8% received oxygen, 1.69% required critical care and 69.4% were readmitted in 12 months. 57.8% of admissions were from the most deprived IMD decile and the average LOS of these patients was longer than the least deprived. The relationship between mean NO_2 exposure and monthly admissions was non-significant. Nearly 60% of admissions were associated with NO_2 exposure exceeding WHO guidelines. These results support improvement in several areas, including follow-up, air quality and health inequality.

Together, these findings emphasise the importance of holistic care for preschool wheeze, considering acute severity, pathophysiology, and environment. If this approach is adopted in research and clinical practice, it could improve management and respiratory outcomes.

Acknowledgements

I would like to thank Professor Paul McNamara, my primary supervisor, for his fantastic support and guidance throughout my MPhil. The research opportunity he has provided has taught me a great amount about the world of research and about myself. I have relished this opportunity to pause my medical studies, and gain an appreciation of the bigger picture. He has remained enthusiastic about my research throughout the year and provided much useful advice and feedback. I have learned a great deal about academic writing from him. I am also very grateful to Dr Dan Hawcutt, my secondary supervisor, who provided me with much support in the transition from clinical medicine to research. In particular, his support with the systematic review has been invaluable. He also helped me appreciate the context of this work and its possible implications.

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COVID-19 Impact Assessment

COVID-19 has had some impact on the work contained in this thesis. Originally, I had planned to collect data from patients being admitted with viral-induced wheeze or asthma over the autumn/winter period of 2020/2021, with use of severity scores to assess them. Due to COVID-19 and the associated social distancing, there were significantly reduced respiratory admissions over this time, meaning that this has not taken place. Instead of this, I undertook additional analysis of the admissions with acute wheeze to AHCH for the period 2015-2020.

Due to COVID-19, the required social distancing and several periods of staff self-isolation, the start of my laboratory investigations was delayed. As a result, I was unable to carry out the primary cell culture and infection that was originally planned. I also did not have time to carry out ELISA, which I had planned to.

The systematic review was unaffected by COVID-19 as this could be completed off-site. Most of the supervision I received was online, with supervisor meetings occurring via Zoom.

Presentations

The work contained within the second chapter was presented as an E-poster at the online ERS Congress in September 2021. The poster was entitled ‘Severity scores published for acute wheeze in preschool children: a systematic review’.

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List of abbreviations

AHCH	Alder Hey Children's Hospital
ALSPAC	Avon Longitudinal Study of Parents and Children
API	Asthma Predictive Index
AQAP	Air Quality Action Plan
AQMA	Air Quality Management Area
AS	Asthma Score
ASS	Asthma Severity Score
AURN	Automatic Rural and Urban Network
BMI	Body Mass Index
BTS	British Thoracic Society
CAES-2	Clinical Asthma Evaluation Score 2
CAS	Clinical Asthma Score
CCU	Critical Care Unit
CDHR3	Cadherin-related Family Member 3
cDNA	Complementary Deoxyribonucleic Acid
CF	Cystic Fibrosis
CO	Carbon Monoxide
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus Disease 2019
DEFRA	Department for Environment, Food and Rural Affairs
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic Acid
DPI	Dry Powder Inhaler
dsRNA	Double Stranded Ribonucleic Acid
EBC	Exhaled Breath Condensate
ED	Emergency Department
EDN	Eosinophil-Derived Neurotoxin
ELISA	Enzyme-linked Immunosorbent Assay

ERS	European Respiratory Society
EVW	Episodic Viral Wheeze
FBS	Foetal Bovine Serum
FENO	Fractional Exhaled Nitric Oxide
GORD	Gastro-oesophageal Reflux Disease
GWAS	Genome-wide Association Study
HDU	High Dependency Unit
HRV	Human Rhinovirus
I:E ratio	Inspiration to Expiration Ratio
ICAM-1	Intercellular Adhesion Molecule 1
ICS	Inhaled Corticosteroids
ICU	(Paediatric) Intensive Care Unit
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IMD	Index of Multiple Deprivation
IQR	Interquartile Range
LDLR	Low-density Lipoprotein Receptor
LFA-1	Leukocyte Function-associated Antigen 1
LPS	Lipopolysaccharide
LSOA	Lower Layer Super Output Area
MDA5	Melanoma Differentiation Associated Gene 5
MOI	Multiplicity of Infection
mRNA	Messenger Ribonucleic Acid
MTW	Multiple Trigger Wheeze
NF-kB	Nuclear Factor Kappa-light-chain-enhancer of Activated B cells
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NO_2	Nitrogen Dioxide

NOS	Newcastle Ottawa Scale
NO_x	Nitrogen Oxides
O_3	Ozone
OCS	Oral Corticosteroids
OPCS	Office of Population Censuses and Surveys
PASS	Paediatric Asthma Severity Score
pBEC	Primary Bronchial Epithelial Cell
PBS	Phosphate-buffered Saline
PCR	Polymerase Chain Reaction
PDT	Passive Diffusion Tube
PEWS	Paediatric Early Warning Score
PFU	Plaque Forming Units
PICO	Population Intervention Comparison Outcome
PM_{10}	Particulate matter diameter <10µm
$PM_{2.5}$	Particulate matter diameter <2.5µm
PRAM	Preschool Respiratory Assessment Measure
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PROM	Patient-Reported Outcome Measure
PRR	Pattern Recognition Receptor
RCPCH	Royal College of Paediatrics and Child Health
RCT	Randomised Controlled Trial
RIG-I	Retinoic Acid Inducible Protein I
RNA	Ribonucleic Acid
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction$_3$
SABA	Short-acting Beta-2 Agonist
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SIGN	Scottish Intercollegiate Guidelines Network

SIW	Severe Intermittent Wheeze
SNP	Single Nucleotide Polymorphism
SO_2	Sulphur dioxide
ssDNA	Single Stranded Deoxyribonucleic Acid
Th	T Helper
TLR	Toll-like Receptor
TNF-α	Tumour Necrosis Factor Alpha
UK	United Kingdom
URTI	Upper Respiratory Tract Infection
VIW	Viral-induced Wheeze
WHO	World Health Organisation

Chapter 1: Introduction

1.1 Background

1.1.1 What is wheeze?

Wheeze is a high-pitched whistling sound that is produced during breathing, most often in the expiratory phase[1, 2]. Wheeze is caused by partial airway obstruction and is associated with increased work of breathing[3]. Poiseuille's law states that airway resistance is inversely proportional to the fourth power of the radius of the airway[2]. Therefore, in preschool children, with relatively small diameter airways, a small absolute reduction in the airway diameter can significantly increase airway resistance[2].

Whilst the exact mechanism of wheeze production has not been completely defined, mathematical models suggest that wheeze is caused by oscillation of the airway walls and airway fluid, which occur at a critical velocity of air flow[4, 5]. The reduced airway lumen diameter in obstructive airway diseases, such as asthma, leads to increased velocity and turbulence of airflow, thus a wheeze is often heard during exacerbations[5, 6]. It should be noted that wheeze is a sign of an underlying condition, not a diagnosis of itself. Current understanding is that wheeze in preschool children is a final common pathway of several pathophysiological processes, which can be difficult to distinguish[7].

Wheeze is a common presentation among young children. Spycher *et al.* analysed the data collected from the Leicestershire cohort study and the Avon Longitudinal Study of Parents and Children (ALSPAC) to investigate the prevalence of wheeze in children[8]. Across both cohorts, it was found that 23% of children had current wheeze at age 2 years and 19% at age 4 years[8]. However, there are often differences in understanding and detection of wheeze in children between

parents and clinicians, with agreement of less than 50% in some cases[9, 10]. Whilst any parent-reported wheeze should be appropriately explored, a wheeze confirmed by a physician carries more diagnostic weight than one reported by parents alone[9, 11]. This finding should be remembered when considering the results of parental surveys for wheezing prevalence and severity[10].

1.1.2 Classification of preschool wheeze

Preschool wheeze is heterogenous, with multiple phenotypes now recognised[12]. Unfortunately, there is widespread confusion regarding the classification and terminology used in this age group[11]. For instance, a recent review article, by Douros *et al.*, listed 22 different descriptors of acute lower respiratory tract infections in infants and young children[13]. The European Respiratory Society (ERS) Paediatric Respiratory Medicine Handbook, updated in 2021, contains a chapter entitled 'Preschool wheezing', which provides a useful overview[14]. It is hoped that this coherent summary will help clinicians to understand the nuances of this broad disease entity.

Two main classification methods for preschool wheeze exist, grouped according to epidemiological and clinical characteristics[2]. Generally, epidemiologists use a hypothesis-free approach for longitudinal analysis of cohorts, often using latent-class analysis[15]. Epidemiological classification aims to help predict the risk of long-term wheeze according to age of onset and other risk factors[12]. This is in contrast to the pre-determined phenotypes applied by clinicians, based on prior history taking, examination and treatment responses of individuals[15].

Epidemiological classification may help to understand the natural history of each preschool wheeze subtype, however it is of limited clinical use and can often only be applied retrospectively[2, 3]. Moreover, these methods are more useful for

application to large groups as the reported patterns may not apply to individuals[16]. However, the asthma risk tools generated from these epidemiological studies may be able to identify those individuals who are unlikely to benefit from long term therapy due to low risk of subsequent asthma, thus reducing unnecessary treatments[17].

A notable example of an epidemiological classification originates from a landmark study from 1995, when the Tucson Children's Respiratory Group (Arizona, USA) classified children into four phenotypes according to the natural history:

- Never wheezed
- Transient early wheeze
- Late-onset wheeze
- Persistent wheeze[12].

More recent cohort studies have suggested the addition of one, or both, of intermediate-onset wheeze and persistent early wheeze phenotypes to the classification[18-20].

The second main classification is based on the clinical symptoms of each patient and their pattern of exacerbations, including identified triggers[7]. This classification is more useful clinically and can be applied on the first meeting of a clinician and patient, using a thorough history and examination[15]. Clinically-orientated classification has multiple benefits including stratification of treatment benefit and prognosis[7].

The most well-recognised clinical phenotypes were proposed in 2008, when the ERS Task Force on Preschool Wheeze recommended that preschool wheeze be subclassified into episodic viral wheeze (EVW) and multiple-trigger wheeze

(MTW)[11]. Notably, this distinction is often not made in routine practice or clinical research[3]. In 2014, the Task Force published an update to the classification based on new evidence[7]. It was acknowledged that the pattern of wheeze in individuals can change over time[21, 22], and with treatment, thus making it difficult to differentiate between the phenotypes of EVW and MTW in some children[7].

EVW is used to describe children who experience wheeze only during viral infections, without interval symptoms[8]. EVW is the most common phenotype seen clinically[23]. This is similar to what has historically been known as 'viral-induced wheeze'. On the other hand, MTW is more similar in presentation to asthma and, in addition to viruses, precipitants may include allergens, exercise and laughter[8, 24]. Symptoms in response to these triggers may indicate airway hyperresponsiveness[23]. For MTW to be diagnosed, a child must have an episode of wheeze in the absence of an infection and/or experience interval symptoms[8, 22].

Phenotype switching is common among preschool children with wheeze[8, 22]. Spycher *et al.* analysed the results of the ALSPAC cohort and found that 22% of those with EVW at age two years who had a classifiable wheeze phenotype at age four years were reclassified as MTW[8]. Conversely, 10% of the children aged two years with MTW had switched phenotypes to EVW by age four[8]. In addition, there is an overlap between the conditions of MTW and EVW and it has been shown that there is poor agreement between paediatrician assessments and diary-based classifications[25].

Another criticism of the ERS classification of preschool wheeze is that a high degree of variation in response to treatment exists between individuals within the same group, meaning that, without a more individualised approach, the classification is of limited value in guiding management[24]. In addition, this

classification does not include the severity or frequency of symptoms, so children with a wide variation of presentations are grouped under each term[24]. Furthermore, it is often unclear which category children should be assigned[26].

However, some argue that this clinical classification is useful for guiding treatment of preschool wheeze. Some studies have reported different treatment responses in each phenotype, with maintenance treatment with low to moderate dose inhaled corticosteroids (ICS) being shown to be largely ineffective in EVW, but more effective in MTW[2, 3]. In addition, the prognosis of the two subgroups of wheezing children may be significantly different. For instance, one prospective cohort study showed the prognosis for children diagnosed with mild EVW at 3 years of age is relatively good, with over two thirds of these children remaining in the least severe groups (symptom-free or mild episodic wheeze) by age 5 years[27]. At the same time, this study suggested that the more severe the phenotype is initially, the worse the long term risk of respiratory disease[27]. Moreover, it was concluded that the mild EVW phenotype is most strongly associated with remission[27].

A prospective observational cohort study of 147 preschool children, split into EVW, MTW and severe intermittent wheeze (SIW) phenotypes, found that children with the MTW and SIW phenotypes exhibited lower plasma concentrations of both Th1 (such as interferon gamma, IFN-γ) and Th2 cytokines (including interleukin 5, IL-5), as well as antiviral substances (such as IFN-β), compared to children with EVW[28]. It should be noted that this study recruited children with severe preschool wheeze only, so may not be fully representative[28]. Despite this, these findings show some apparent immunological differences

between the three phenotypes of wheeze identified, thus showing potential for better targeted treatment strategies for these children[28].

Many interventional randomised controlled trials (RCTs) in the preschool age group do not use a standard definition for inclusion and does not attempt to distinguish phenotypic groups, instead using broad terms such as 'recurrent wheeze' or 'reactive airway disease'[13]. As a result, it is difficult for those interpreting this research to ascertain which phenotypes have been included, and therefore assess the transferability of findings[13]. For instance, if all subgroups of preschool wheeze are analysed together, the results may be different compared to if subgroup analysis for each phenotype is performed[13]. This emphasises the need for more consistent and precise use of definitions in this patient cohort, with international consensus.

1.1.3 The importance of preschool wheeze

Preschool wheeze is a significant cause of medical attendances in both primary and secondary care[29]. Of all paediatric hospital admissions (aged 1-16) in the UK for acute wheeze or asthma exacerbations between 1998-2005, approximately 75% were for children less than 5 years old[30, 31]. This trend is supported by figures released by the USA Centre for Disease Control, which showed that for the years 2004-2005, there were significantly more ambulatory and emergency department (ED) visits for acute asthma, per unit population, in children aged 0-4 years, than children aged 5-10 or 11-17 years[32]. For instance, there were 165.1 ED visits per 10000 children aged 0-4 years, compared to 102.6 per 10000 in children aged 5-10 years, and 59.7 per 10000 in the 11-17 years age group. Moreover, a recent population-based cohort study of over 1 million preschool children in the UK

showed that, over a median follow-up period of 2 years, 15.8% had an ED attendance, and 13.9% were admitted to hospital, for a respiratory disorder[33].

Preschool wheezing disorders accrue significant costs to the National Health Service (NHS) in the UK, estimated at £53 million in 2003[34]. The largest proportion of healthcare costs are in primary care (65.2% of total)[34]. Moreover, the prevalence of wheeze in children aged 2-5 years has increased in recent years, so the financial impact is likely to also have increased[33, 35]. In addition, preschool wheeze can have a significant negative impact on the health-related quality of life of affected children[36].

1.1.4 Outcome measures for preschool wheeze

Patient-reported outcome measures (PROMs) are instruments that are used to measure outcomes of medical conditions that are important to each patient[37]. PROMs are an important part of a transformation of clinical medicine, aiming to deliver patient-tailored care. There have been many disease-specific outcome measures developed in recent years, but few of these are specific to paediatrics[38]. There have been recent attempts to develop and validate a PROM for use in preschool wheeze[37-39] The 'wheeze and me' assessment tool has been developed and provisionally validated using the caregivers of 15 children with preschool wheeze[39]. Recruitment of 500 caregivers is currently taking place, which should provide a substantial dataset to more thoroughly validate this instrument[39]. If this tool, or an alternative PROM, is successfully validated, it could provide a useful endpoint for clinical research and for guiding treatment priorities[39].

1.2 Aetiology of preschool wheeze

There are several factors associated with wheeze exacerbations, but viruses are the major cause[21]. Episodic viral wheeze is common in young children, and viruses

are also the most common cause of multiple trigger wheeze exacerbations[8, 21]. In fact, viruses are implicated as the aetiological factor in 80-90% of wheezing episodes in early childhood (**Figure 1.1**)[40, 41]. Other risk factors for exacerbations include exercise, inhaled allergens and environmental irritants, including air pollutants[42].

The most common causes of episodic viral wheeze are human rhinovirus (HRV), respiratory syncytial virus (RSV), human metapneumovirus, parainfluenza virus, adenovirus and coronavirus[2, 43]. RSV infection predominates in infants, in whom it causes bronchiolitis, whereas rhinovirus-associated wheezing is more common in older hospitalised children, with the transition occurring at approximately 12 months[44, 45].

It must be noted that the pathophysiology of RSV bronchiolitis is significantly different from acute preschool wheeze caused by RSV. In bronchiolitis, the respiratory epithelium is infected, leading to increased mucus production, cell death and airway oedema[46]. As a result, there is airway obstruction, leading to wheeze[46]. There is not airway hypersensitivity and smooth muscle contraction, as observed in preschool wheeze and asthma[3]. As a result, the common treatments which act to reduce bronchospasm, such as inhaled salbutamol, are not efficacious[3, 46].

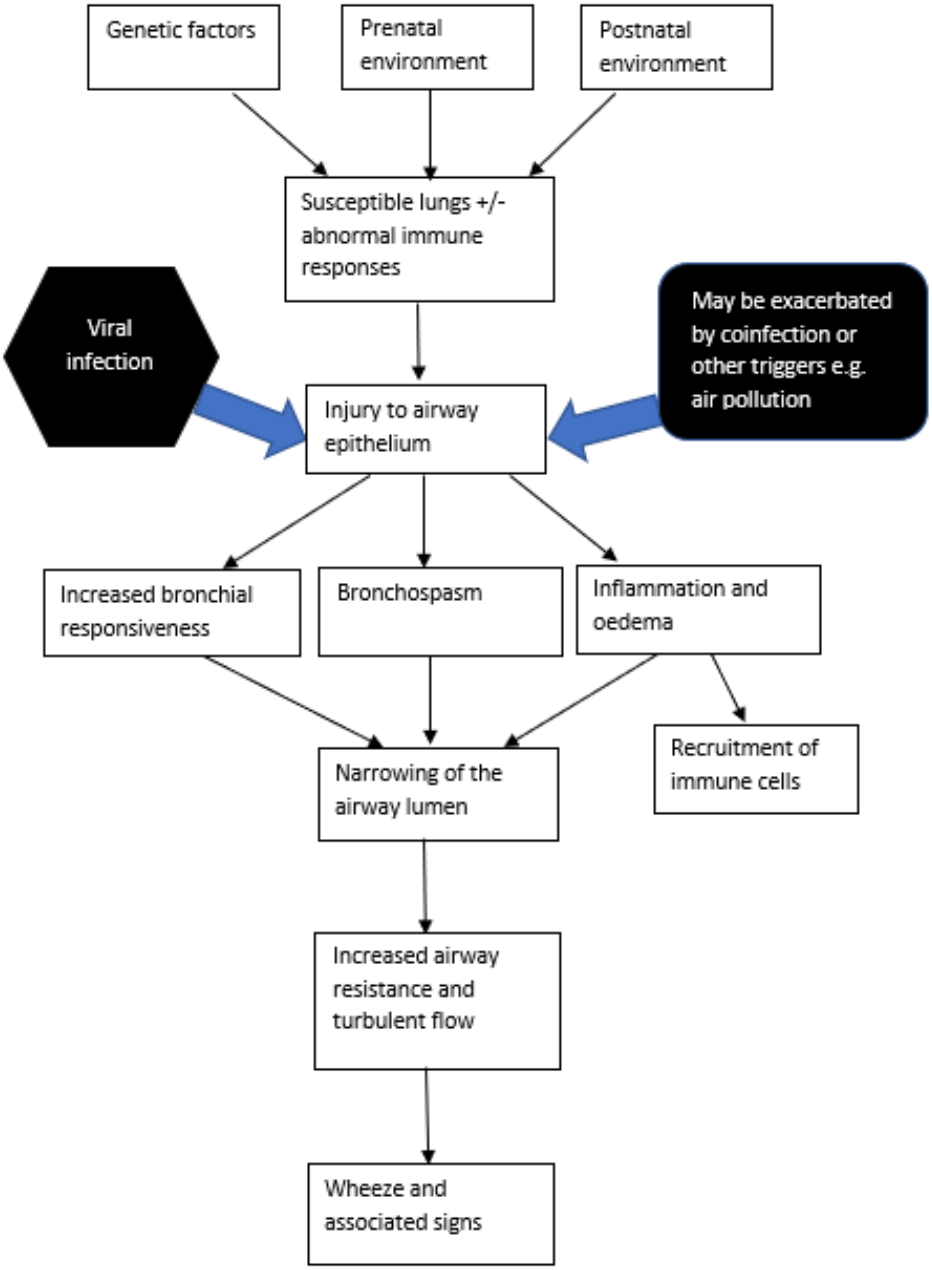


Figure 1.1 – Mechanism of viral-induced wheeze[1, 2, 47-49](in multiple trigger wheeze, this process may occur in the absence of viral infection).

1.2.1 Human rhinovirus (HRV)

HRV belongs to the *Picornaviridae* group and is a single-stranded RNA virus[50]. Picornaviruses are composed of an protein capsid with an icosahedral architecture, encasing the RNA genome[51]. HRV reproduces every 6-8 hours, producing up to 100000 virus particles per cell[52]. Currently, over 160 different types of rhinovirus which have been discovered, split into three species (A, B and C), each with different genomic features[53, 54].

HRV is thought to be one of the most prevalent pathogens affecting humans, with rhinovirus implicated in approximately half of all common cold cases, as well as some cases of bronchiolitis, otitis media, rhinosinusitis and pneumonia[50, 55, 56]. In addition, HRV plays an important role in asthma exacerbations[57]. In a study of 9 to 11 year old children diagnosed with asthma, 80% of wheezing children had a positive viral test result, with approximately two thirds attributed to HRV[58]. Moreover, a longitudinal study of preschool children suggested that each child was infected with picornavirus six times annually[59]. Whilst the sample size was only fifteen, it gives an indication that rhinovirus infection is very common among preschool children[59].

It used to be thought that rhinovirus infection was exclusive to the upper airways, until more recently HRV has been found replicating in the lower respiratory tract[60]. In non-asthmatic individuals, rhinovirus infections are often associated with upper respiratory tract symptoms, such as rhinorrhoea[61]. On the contrary, asthmatic patients more often experience lower respiratory tract symptoms such as cough, dyspnoea and wheeze[61]. There are multiple mechanisms at play in the generation of symptoms, such as shedding of airway epithelium and airway oedema, which interact to cause airway obstruction and thus wheeze[62].

Analysis of UK epidemiological data has shown that HRV infections peak during Autumn and Winter, and they are most common in children under 5 years[63]. In addition, it is not unusual for HRV to coinfect with another virus, such as parainfluenza or RSV[63]. HRV can be diagnosed using reverse transcriptase polymerase chain reaction (RT-PCR); which is relatively sensitive and specific[57].

A study of 3898 adult patients in Southampton during the early COVID-19 pandemic compared the circulation patterns of HRV between March-September 2019 and 2020. It was shown that the circulation of HRV remained low from March until September, despite the easing of lockdown measures, suggesting that the return of children of schools had a significant impact on HRV transmission (**Figure 1.2)**[64]. However, these figures may not be fully representative of the cases in children. In contrast, a nationwide viral epidemiological study undertaken in Finland, found the reopening of schools had no impact on the incidence of any respiratory infections, including HRV[65]. Conversely, data from Australia has showed that, when compared to other respiratory pathogens, such as RSV, HRV transmission has been relatively unaffected by the restrictions associated with the COVID-19 pandemic[66]**.** These conflicting findings emphasise the need for more

research into HRV transmission, especially in children.

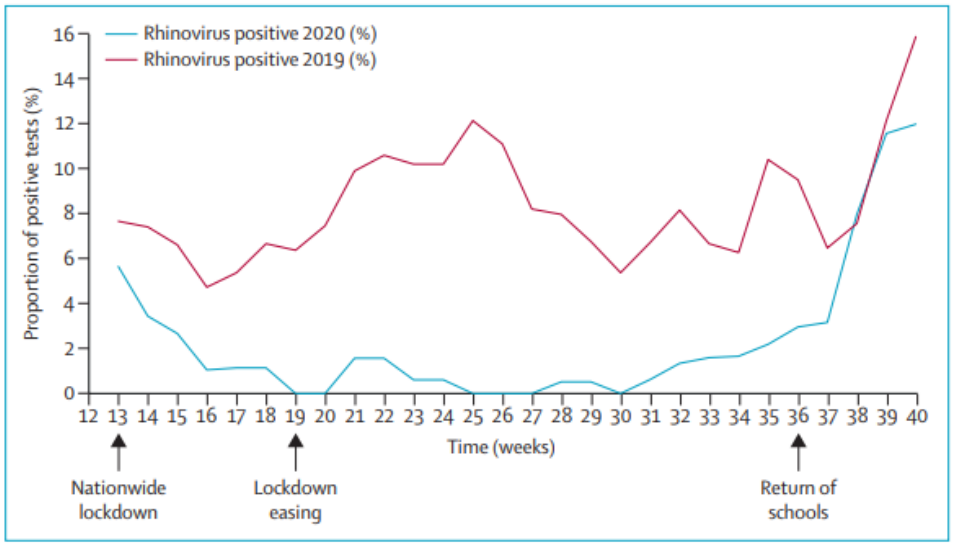


Figure 1.2 – Proportion of adult medical patients in Southampton who tested positive for HRV for 2019 and 2020. Data points represent a 2 week rolling average[67]

1.2.1.1 Rhinovirus receptors

Rhinoviruses use three different cell membrane glycoproteins located on the surface of host cells to enter human respiratory epithelium[53]. The receptors which have been identified are: intercellular adhesion molecule 1 (ICAM-1), low-density lipoprotein receptor (LDLR) family members and cadherin-related family member 3 (CDHR3)[53]. These receptors each bind to different virus groups, have different structures and mechanisms of action[53].

ICAM-1 was discovered in the late 1980s, when three research teams independently identified it[68-70]. This receptor is used by most HRV-A and B serotypes to enter respiratory epithelial cells[50]. The discovery of ICAM-1 was closely followed 5 years later by the detection of the low-density lipoprotein (LDL) receptor family[53, 71]. Twelve known HRV-A viruses belong to the minor group that use these receptors[54, 71]. At least three LDL receptors are able to bind to HRV[53]. The group of serotypes that use ICAM-1 is called the 'major group', whilst a small

proportion of HRV-A serotypes use LDL receptors instead, named the 'minor group'[53, 72].

ICAM-1 belongs to the immunoglobulin family of proteins, responsible for cell adhesion, and it is expressed by multiple cell types during stress or inflammation[53]. In addition to allowing cell attachment for major group rhinoviruses, the ICAM-1 receptor allows the release of viral RNA into the host cell[73]. Unusually, HRV infection is thought to increase expression of its own receptor[74]. It has previously been shown that major group rhinovirus infection can increase the expression of ICAM-1 on cell surfaces, via increased transcription of mRNA, mediated by upregulation of NF-κB (Nuclear Factor Kappa-light-chain-enhancer of activated B cells).[74, 75].

The upregulation of ICAM-1 by airway epithelium could be responsible for the influx of inflammatory cells into the epithelium, and for worsening the inflammatory landscape[74]. Therefore HRV infection can cause a self-perpetuating cycle of inflammation, and allow HRV infection to spread via newly-expressed receptors[52]. This may be pivotal in the pathogenesis of viral-induced wheeze or asthma exacerbations. It is thought that if the expression of ICAM-1 could be reduced, this could mitigate the proinflammatory pathways and inflammatory cell influx associated with rhinovirus-induced wheeze exacerbations[74].

Rhinovirus C was discovered in 2006, owing to advancement in molecular typing methods, using polymerase chain reaction[55]. Unlike rhinovirus A and B, RV-C is resistant to growth in traditional tissue culture conditions[76]. RV-C has been linked to more severe infections in preschool children, of both the upper and lower respiratory tracts, than the other serotypes[77]. Moreover, asthma exacerbations have been reported as more severe in children infected with RV-C[78].

HRV-C has one known receptor, known as cadherin-related family member 3, which is thought to enhance binding and replication of RV-C[53, 79]. Receptors of the cadherin-related family use calcium ions to communicate between networks of cells, although the purpose of several members of the family is as yet unknown[54, 76, 79]. It has been found from cell culture that HRV-C spread between cells is reliant on the level of CDHR3 expression, and so this may also be true in natural HRV-C infections[76]. Interestingly, CDHR3 has been proposed by a genome-wide association study (GWAS) to be linked to susceptibility for asthma in children aged 2-6 years, especially for recurrent, severe exacerbations[80].

A recent study suggested that a single nucleotide polymorphism or SNP (rs6967330) in the CDHR3 receptor, that converts cysteine to tyrosine at position 529 in the polypeptide chain, is linked to increased binding of HRV-C[54, 79]. A suggested reason for this is the difference in stability of the cell surface, leading to easier access of HRV-C into airway cells expressing the CDHR3 receptor[53, 76]. Whilst this study was in vitro, if this association was also found in vivo, this SNP could be a determinant for more severe HRV-C infections[79]. The Tyr529 variant is uncommon, but acts in a dominant fashion in producing the phenotype[76].

It is thought that CDHR3 is predominantly found within intercellular junctions of the cell membrane[54]. In this location it is protected from contents of the airways, including viruses[54]. In the airways of those affected by asthma, there is often a reduced epithelial barrier function[54]. This may be due to the airway inflammation underlying asthma or an associated genetic defect[54]. It has been proposed that the poor barrier function in asthma increases the exposure of CDHR3 to the airway lumen, thus increasing the risk of HRV-C infections[54].

Whilst the exact mechanisms of rhinovirus pathogenesis are not entirely clear, it is understood that infection of epithelial cells with rhinovirus can further impact upon the epithelial barrier function, primarily by disruption of tight junctions between cells[61]. All rhinovirus serotypes enter host airway cells from the apical aspect, via receptor-mediated endocytosis (**Figure 1.3**)[53, 60]. Once endocytosis occurs, the low pH environment within the endosome allows translocation of the viral RNA into the intracellular fluid[81].

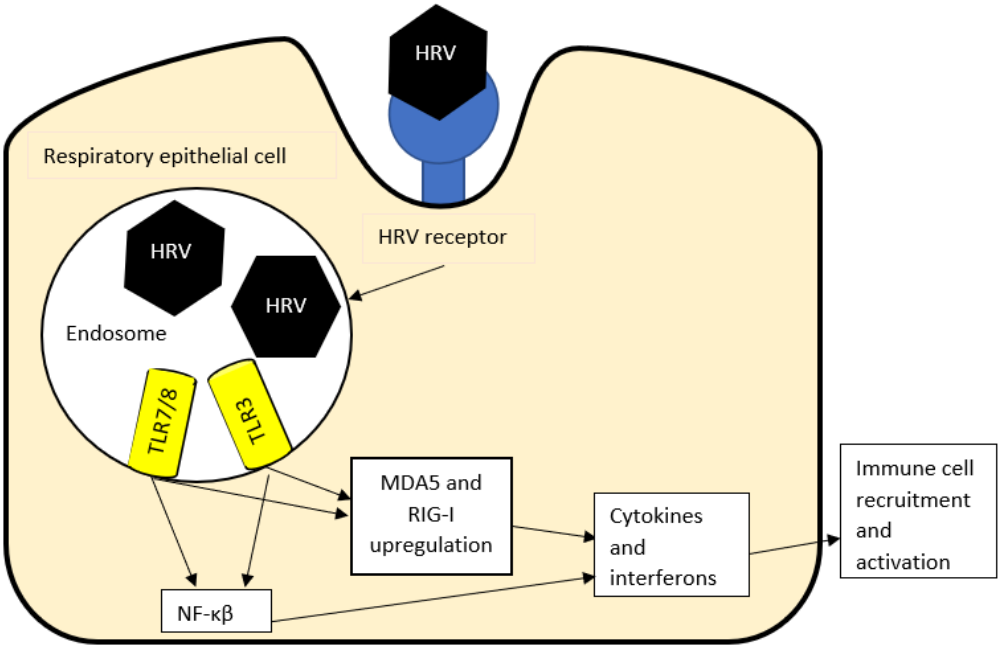


Figure 1.3 – Simplified immune response of respiratory epithelial cells to human rhinovirus (HRV) infection[82-84]*. HRV binds to its corresponding receptor (ICAM-1, LDLR or CDHR3), and enters the cell via endocytosis*[83]*. Once HRV is inside epithelial cells, it is uncoated due to the relative acidity and its genetic material is recognised by pattern recognition receptors (PRRs), thus inducing inflammatory responses*[83, 84]*. In particular, HRV double stranded (ds)RNA is recognised by toll-like receptor 3 (TLR3), which in turn leads to upregulation of other PRRs, such as melanoma differentiation associated gene 5 (MDA5) and retinoic acid inducible protein I (RIG-1)*[82, 84]*. It is also thought that TLR7/8 binds to single stranded (ss)RNA*[84]*. These TLRs are thought to induce release of interferons and proinflammatory cytokines, including IFN-γ and IL-8, as well as activation of the NF-κβ pathway*[83, 84]*. These cytokines and interferons then recruit and activate immune cells, including neutrophils, eosinophils and B and T lymphocytes*[82, 83]*. Activation of the NF-κβ pathway is thought to increase the expression of ICAM-1 by positive feedback in major group HRV infection*[74]*.*

HRV is not cytotoxic and, unlike some other viruses, generally does not cause destruction of the monolayer during infection[85, 86]. Therefore, it is thought it exerts its effects on the respiratory epithelium by altering the biology of the epithelial cells[86, 87]. It has also been hypothesised that the immune response to HRV infection, not only the direct effects of the viral infection, can be significant in the pathogenesis of viral exacerbations of wheeze[57].

Cytokines are hormonal molecules responsible for most of the communication within the immune system[88]. Cytokines are divided into two main groups, according to the type of T helper, or CD4, cells that they interact with, namely Th-1 and Th-2 associated cytokines[88]. Th-1 group cytokines are associated with autoimmune responses, and intracellular parasitic immune responses[88]. Conversely, Th-2 type cytokines participate in the immune response to extracellular parasites[89], and work with eosinophils to promote atopy and immunoglobulin (Ig)E release[88]. These responses should be carefully balanced for optimal immune function, nevertheless the Th-2 associated pathway usually predominates in asthma[88].

The pathogenesis of asthma has been proposed as a simplified two-step model[90]. Firstly, sensitisation to a particular aeroallergen occurs, which is accompanied by development of antigen-specific immune cells, mainly of Th-2 lineages[90]. Secondly, the allergic inflammation underpinned by the Th-2 cells, and associated cytokines and growth factors, is targeted to the lower respiratory tract[90]. The Th-2 like cytokines involved in the pathogenesis of asthma include IL-4, -5 and -13[90]. The exact roles of each cytokine have not been fully elucidated, but it has been postulated that the Th-2 cell inflammation of the airways is driven by increased secretion of proinflammatory cytokines and reduced expression of

immunoregulatory cytokines, leading to an imbalance of cytokines[91]. It is thought that this pattern of cytokine secretion is the cause of hyperresponsiveness in asthmatic airways[90].

It has been shown that rhinovirus infection under experimental conditions is associated with increased hypersensitivity of the airways[57, 92, 93]. The roles of immune cells in viral exacerbations of asthma or wheeze are only partially understood[74]. Eosinophils and bronchial mucosal lymphocytes both play an important role in the response to HRV infection, as shown by a publication by Fraenkel and colleagues, which reported the findings of bronchoscopies in 17 patients following HRV-16 infection (including 6 atopic asthmatics)[94]. However, these findings may not be fully transferrable to children with asthma, or preschool wheeze, as these presentations may be associated with different pathophysiology[3, 94].

1.2.1.2 Rhinovirus treatments

The only available treatments for HRV are for symptomatic relief, and do not act on the specific inflammatory pathways[53]. The recent discoveries of HRV receptors, and the corresponding inflammatory pathways, bring hope for targeted treatments for rhinovirus infection. There are multiple hurdles that must be overcome before an effective treatment for rhinovirus infection can be obtained. These include: the high mutation rates of HRV RNA (increasing the risk of drug resistance), and the current lack of a reliable point-of-care diagnostic test[53]. If these problems are overcome, it is possible that novel treatments blocking the three HRV receptors could be effective in stopping receptor-mediated endocytosis, and thereby infection, of respiratory epithelium[53] (see **Figure 1.4**).

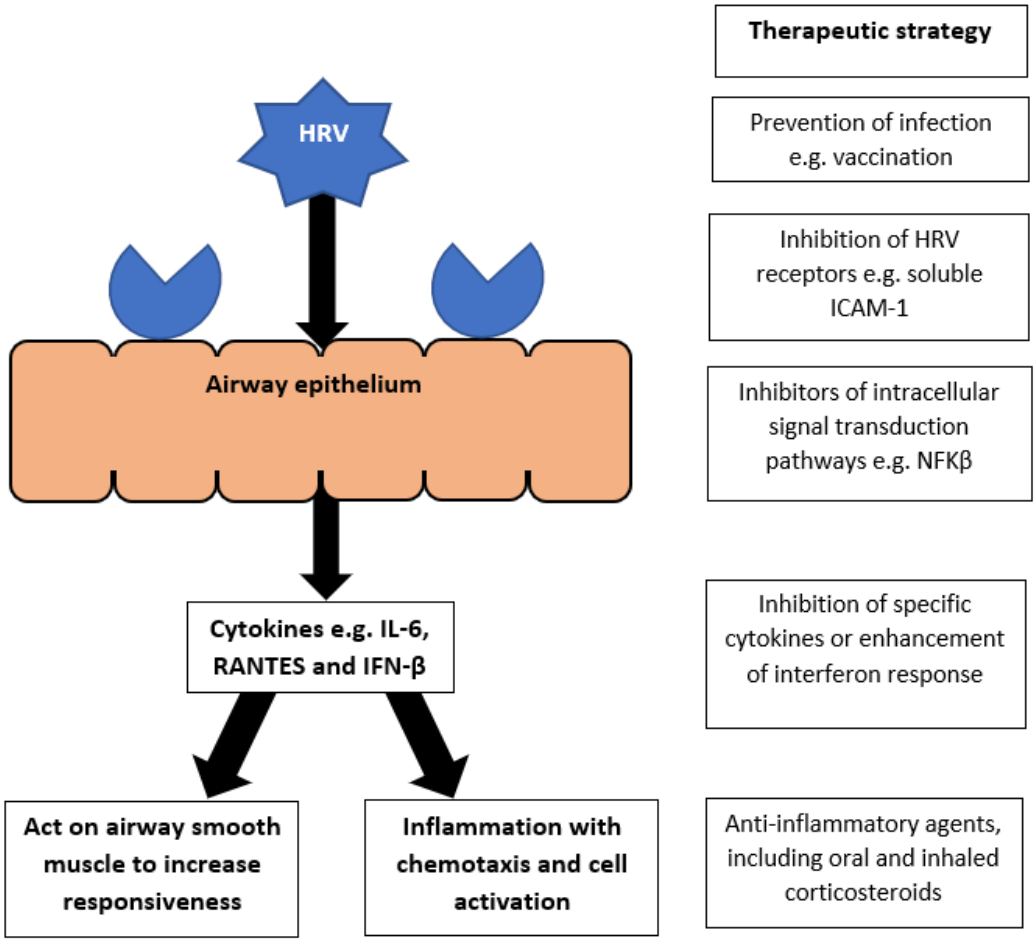


Figure 1.4 Mechanism underlying HRV-induced wheeze and asthma exacerbations, including some potential therapeutic targets. Adapted from Gern et al., 1999[57], with reference to Jackson et al., 2010[95]

One such potential therapy is an anti-human ICAM-1 antibody that can reduce inflammatory cell recruitment, pro-inflammatory cytokine release and prevent entry of HRV into respiratory epithelium[96]. There have also been several studies investigating whether existing medications are able to inhibit rhinovirus infection of primary respiratory cells, as well as the associated effects on cytokine release[91, 97-99]. For instance, one study investigated the impact of formoterol and budesonide on the cytokine response of primary tracheal epithelial cells when infected with RV14, a major group HRV[98]. The results showed that combination therapy, with formoterol and budesonide, was associated with reduced cytokine

release, including IL-6 and IL-8, as well as reduced concentration of ICAM-1.[98] Similar results have also been found with carbocisteine, a mucolytic medication, in human tracheal epithelial cells[91]. Whilst this is an avenue of great opportunity, significant work is needed to transform these treatments from bench to bedside.

1.2.2 Other viral causes of preschool wheeze

There is limited evidence concerning severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the coronavirus behind the COVID-19 pandemic, as a potential trigger of preschool wheeze. However, most population studies have found coronaviruses to act as coinfections rather than solo pathogens[100]. COVID-19 induced episodes of viral wheezing are rare, and those reported are often mild[101]. In addition, a survey of 174 European centres showed that asthma in children is not a significant risk factor for severe COVID-19, although it has been linked to a relatively high rate of hospital admission[102]. Interestingly, a recent mathematical model has suggested that HRV infection reduces the replication of SARS-CoV-2, most likely by triggering an interferon response[103].

Importantly, the indirect effects of the 'UK lockdown' on child health may have been more significant. It has been reported that UK paediatric emergency attendances have been significantly reduced during the COVID-19 pandemic, as a result there may have been some delays in preschool wheeze treatment, or a preponderance for severe wheeze presentations, since March 2020[67, 104]

More simply, as well as reduced presentations, it may be the case that have been fewer cases of acute wheezing since March 2020. For example, a recent Italian study of 85 children aged 2-6 years with persistent wheeze showed that, during the lockdown period, this population showed significant clinical improvement[105]. More specifically, the families of the children reported fewer ED visits, reduced

day and night time symptoms, fewer episodes of wheeze and reduced medication use (both inhaled bronchodilators and oral corticosteroids)[105]. Proposed reasons for these improvements are: reduced viral transmission due to home-schooling, reduced air pollution and limited exposure to aeroallergens[105]. However, potential confounding factors, such as the recognised season variation in preschool wheeze exacerbation, should be considered[105]. The Italian lockdown that was studied by Ullmann *et al.* took place between March and June 2020[105]. It must be noted that there is a tendency for acute paediatrics wheeze exacerbations to be more common in the Winter and improve through the summer, with a trough in June/July[105, 106]. In addition, it is common for some children to gradually have reduced symptoms over time, regardless of external exposures[105]. Therefore, there may be difficulty in differentiating the effects of lockdown from the natural history of the disease[105].

Due to the lockdown, children in the UK have not been exposed to respiratory viruses as much as usual. This means that there are large numbers of 'virus-naïve' children in the community. It is feared that once lockdown is eased there will be a tidal wave in hospital admissions with bronchiolitis and preschool wheeze in children due to respiratory viruses.

1.3 Risk factors for preschool wheeze

Several risk factors for preschool wheeze have been reported. These factors can be grouped into genetic influences, prenatal factors and postnatal exposures (**Figure 1.1**)[23]. A recent GWAS has identified multiple genes which may be associated with childhood asthma, but few of these have been confirmed[1]. An exception is the ORMDL3 gene, which has been reproducibly linked to asthma susceptibility[107]. However, the pathogenesis of preschool wheeze and asthma is

complex. Each gene has a small effect, and it must be considered alongside the environmental exposures of each child[1, 108].

Prenatal factors, such as intrauterine growth restriction, have been associated with lung dysfunction in later life[109]. Maternal smoking during pregnancy has also been linked to smaller intrapulmonary airways in childhood[12]. Postnatal exposures can also have an impact on the risk of preschool wheeze. For example, maternal cigarette smoking is linked to increased risk of both transient early wheeze and persistent wheeze[12]. Other postnatal exposures implicated in preschool wheeze include bacteria, viruses and air pollution[23].

Bronchiolitis has been recognised as a major risk factor for development of preschool wheeze and/or asthma[110]. A birth cohort study of over 600000 English children, published in 2019, suggested that bronchiolitis requiring hospital admission is linked to a 3-5 times increased risk of later admissions for respiratory illness (acute wheeze, asthma or respiratory tract infection)[111]. In addition, the results indicated that approximately 20% of children who were admitted to hospital for bronchiolitis treatment have at least one more hospital admission for a respiratory illness by the age of 5 years[111]. Whilst the association of bronchiolitis and preschool wheeze is widely accepted, this relationship may not be causal, but instead be because individuals with atypical immune responses, lung development or lung functioning may be more at risk of both syndromes[112].

Severe RSV bronchiolitis can be prevented by the monoclonal antibody palivizumab[113]. A double-blind placebo-controlled trial performed by Blanken *et al.* showed that monthly administration of palivizumab can reduce the overall proportion of infants with recurrent wheeze by 10% (21% vs. 11%, p=0.01)[113]. The results of this landmark study provide convincing evidence that RSV infection is an

important player in the pathogenesis of recurrent wheeze during the first year of life, although this study did not follow up children into the preschool age group[113].

1.3.1 The effect of deprivation on childhood wheezing illness

There have been several studies into the effects of deprivation on the prevalence of wheeze in children, with conflicting results. A prominent longitudinal cohort study in New Zealand published in 2004 showed that, among the study cohort, childhood socioeconomic status was not related to the prevalence of asthma[114]. Conversely, a systematic review published in 2015 concluded that there is an association between asthma and wheeze prevalence in children and lower socioeconomic position, but the factors responsible were not fully investigated[115].

On the other hand, it must be noted that the representative UK Millennium Cohort Study revealed that if the results were adjusted for the confounding factors of breastfeeding and maternal smoking during pregnancy, the socioeconomic inequalities in childhood wheeze were removed[116]. These inconsistent results show that further investigation of this relationship is needed, as well as quantification of any confounders or contributory factors.

1.3.2 The impact of air quality on preschool wheeze

Another important environmental factor implicated in the prevalence and severity of wheezing illness in preschool children is air pollution. There are World Health Organisation (WHO) guidelines for the acceptable levels of air pollution[117]. These guidelines provide worldwide recommended levels of exposure for multiple pollutants, including nitrogen dioxide (NO_2), sulphur dioxide and particulate matter[117] (see **Table 1.1**). In recent years, the considerable impact of poor air quality on children has been highlighted. A notable development was UNICEF releasing a landmark report in 2016 entitled 'Clear the air for children'[118]. This

report included some powerful data and graphics showing both short and long-term effects of air pollution on children[118].

Air pollutant	WHO Air Quality Guidelines
Particulate matter diameter <2.5µm (PM2.5)	24 hour mean: 25 µg/m^3 Annual mean: 10 µg/m^3
Particulate matter diameter <10µm (PM10)	24 hour mean: 50 µg/m^3 Annual mean: 20 µg/m^3
Nitrogen dioxide (NO_2)	1 hour mean: 200 µg/m^3 Annual mean: 40 µg/m^3
Sulphur dioxide (SO_2)	10 minute mean: 500 µg/m^3 24 hour mean: 20 µg/m^3
Ozone (O_3)	8 hour mean: 100 µg/m^3

Table 1.1 – World Health Organisation Air Quality Maximum Recommended Levels for Air Pollutants Implicated in Adverse Respiratory Health Effects[117]

A 2019 meta-analysis of data from 18 European countries concluded that a significant percentage of cases of asthma in children (aged 1-14 years) may be caused by air pollution[119]. The components studied were NO_2, black carbon and particulate matter[119]. It was found that if the WHO guidelines for NO_2 and particulate matter were met, 0.4% and 11% of new cases of asthma in children could be prevented, respectively, showing the significant impact of air pollution on asthma incidence[119]. In addition, a time-series and case-crossover analysis performed in Australia, showed that children are more susceptible to the effects of air pollution on the risk of hospitalisation for acute asthma[120].

1.3.3 Ethnic differences in preschool wheeze in the UK

In recent years, there has been reporting of health inequalities according to ethnicity across many areas of medicine. In fact, a 2018 whitepaper by Public Health England was entitled 'Understanding and reducing ethnic inequalities in health', and concluded that reporting of ethnicity and corresponding inequalities should become widespread[121]. The results of the Millennium Cohort Study have been analysed to explore ethnic differences in the prevalence of preschool wheeze or asthma[122]. This study yielded some interesting results, for instance at age 3 years 8.7% of Bangladeshi children, 19.4% of White children, and 25.5% of Black Caribbean children had experienced recent wheeze[122]. Most of these differences were eliminated by adjusting for socioeconomic and cultural factors, as non-White groups were more likely to be disadvantaged (except Indians)[122]. However, it shows that it can be valuable to consider the demographic profile of patients, to ensure that inequalities can be identified and reduced[122].

1.4 The diagnosis and assessment of acute preschool wheeze

1.4.1 Differential diagnosis

There is a wide list of differentials for wheeze in a child of preschool age (1-5 years). It can be challenging to make a definitive diagnosis in preschool children with wheeze as it can be challenging to perform lung function tests, and the biomarkers associated with the underlying inflammation are poorly defined[123]. The two most common differential diagnoses for preschool wheeze are asthma and bronchiolitis (**Figure 1.4**)[1]. Other possible differentials include: gastro-oesophageal reflux disease (GORD), cystic fibrosis (CF), chronic rhinitis, immunodeficiency, foreign body aspiration and anatomical abnormalities[3].

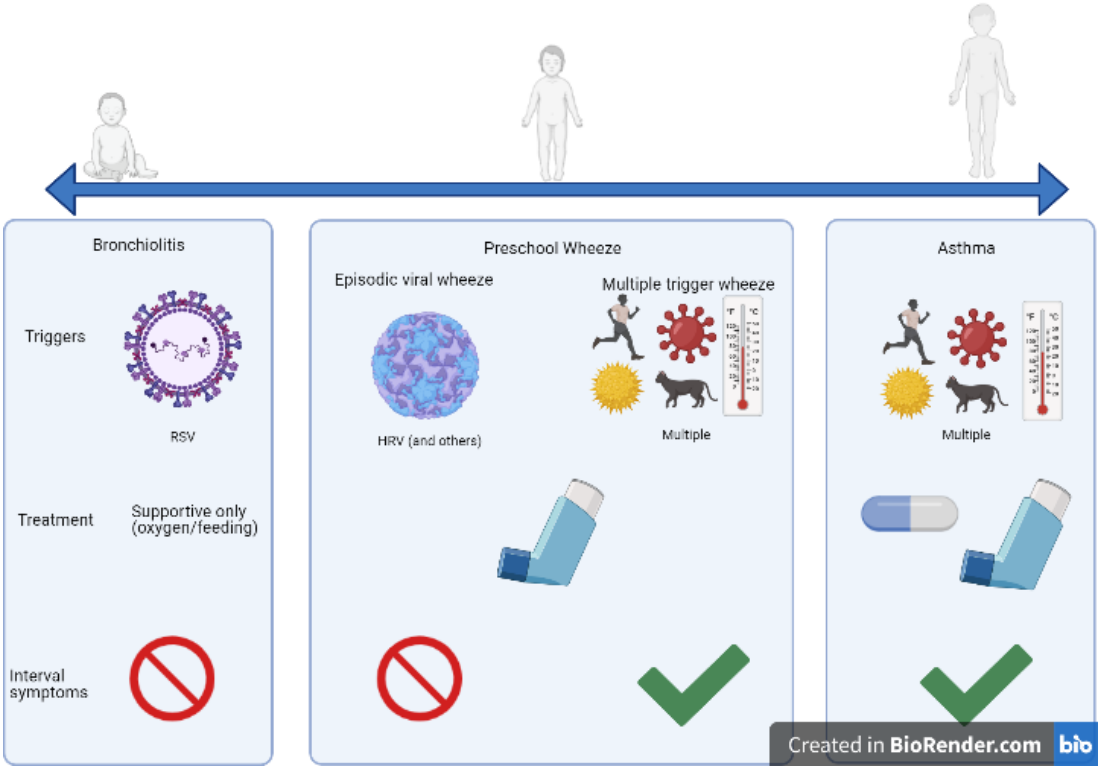


Figure 1.5 – Distinguishing features of wheezing illness in children[3, 7, 23].

Created with BioRender.com[124].

Bronchiolitis is a common acute lower respiratory tract infection that usually occurs in infants, and 90% of children are affected by 2 years[1, 125]. In approximately 75% of cases, respiratory syncytial virus is the cause[126]. It follows a similar seasonal pattern to other paediatric wheezing illness, with a peak incidence in autumn and winter[1]. Bronchiolitis is managed using supportive therapy as, unlike asthma, it responds poorly to corticosteroids and bronchodilators[1].

Asthma presents in a similar way to preschool wheeze, with shortness of breath, cough, chest tightness and chest pain[127]. Asthma is more similar to the MTW phenotype than EVW. For instance, asthma and MTW exacerbations can both be triggered by a number of factors including viruses, cigarette smoke, animal dander

and house dust mite[128]. In addition, children with asthma and MTW have ongoing airway inflammation during times of minimal symptoms, unlike those with EVW[129]. Another similarity between asthma and preschool wheeze is the mechanism, which involves a combination of airway inflammation and bronchial hyperresponsiveness[127, 128]. However, it has been suggested that the underlying pathophysiology may differ, with asthma showing a predominance for eosinophils and mast cells, whereas preschool wheeze has been linked to other inflammatory cells, predominantly neutrophils[130].

On the other hand, an important difference between asthma and preschool wheeze is the response to therapy. For example, OCS are routinely used in asthma exacerbations but the evidence for their use in acute preschool wheeze is equivocal[131, 132]. Additionally, due to the age of the children affected, some investigations, such as lung function tests, may not be possible in preschool wheeze but routinely used in school-age asthma[123]. The long-term prognosis also differs significantly between these syndromes, with preschool wheeze (especially EVW) more likely to remit than asthma[26].

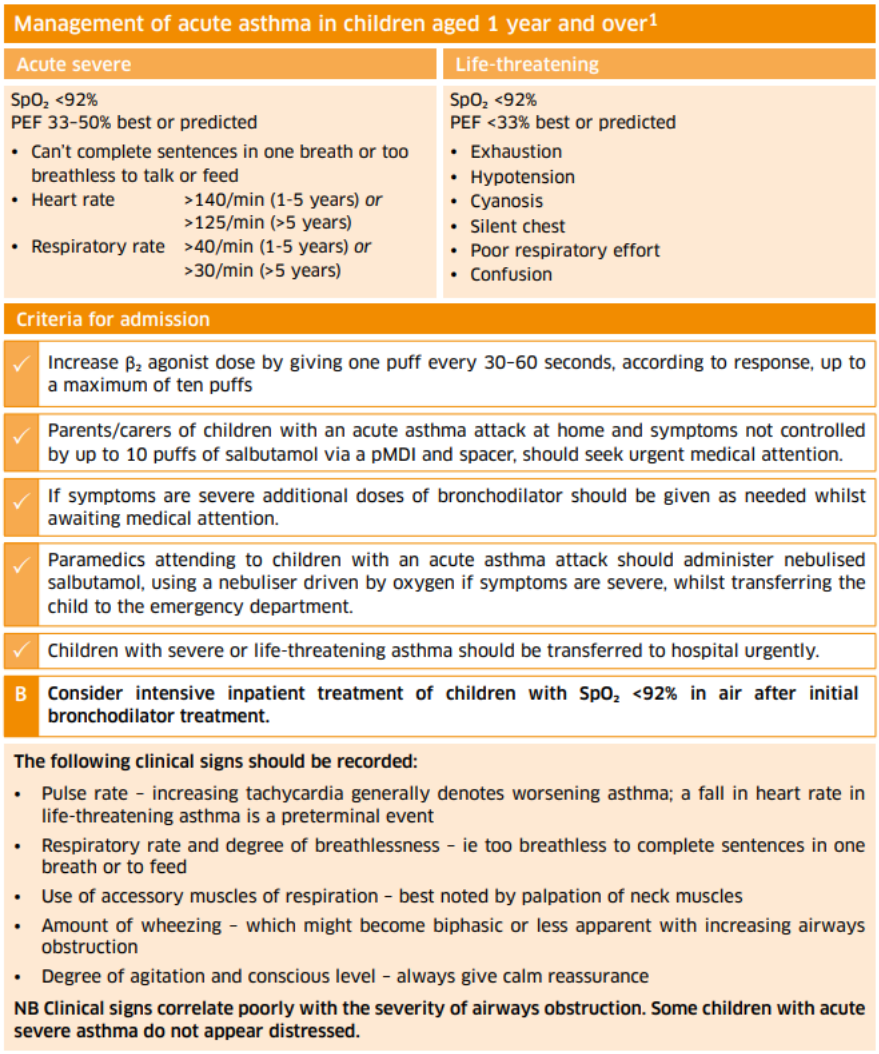
It is important to remember some children experience a physiological wheeze during normal development[3]. Most children with preschool wheeze do not benefit from investigation, which can cause anxiety in children and parents alike[2]. Some red flags which may prompt investigations include: symptoms existing from birth, chronic wet cough, severe airway obstruction and signs of systemic disease[3, 23].

1.4.2 Presentation of acute wheeze in preschool children

The presentation of preschool wheeze is similar to that of asthma, with dyspnoea, wheezing, chest tightness, cough and/or tachypnea[42]. These signs may be accompanied by other markers of severity, such as tachycardia, or signs of

respiratory distress, such as accessory muscle use, poor feeding or limited speech[42]. Signs indicating a life-threatening exacerbation include cyanosis, confusion, exhaustion, hypoxia and silent chest[133].

The National Institute for Health and Care Excellence (NICE) guidelines 'Viral-induced wheeze/infective exacerbation of asthma' provide a useful summary of the available evidence for the assessment of acute preschool wheeze[133]. They recommend categorising exacerbations into moderate, acute severe or life-threatening groups, using a number of clinical parameters[133]. The Scottish Intercollegiate Guidelines Network (SIGN) and British Thoracic Society (BTS) guidelines for 'Management of acute asthma in children aged 1 year and over' use similar categories and can also guide assessment[134], **see Figure 1.6**. The criteria for admission are also a useful resource for clinicians[134]. For children admitted to hospital, regular monitoring of clinical condition and vital signs should be undertaken[133]. Children should receive follow up within two days of hospital discharge, or within two days of presentation (if not admitted)[133].



Management of acute asthma in children aged 1 year and over[1]

Acute severe	Life-threatening
SpO_2 <92% PEF 33–50% best or predicted • Can't complete sentences in one breath or too breathless to talk or feed • Heart rate >140/min (1-5 years) *or* >125/min (>5 years) • Respiratory rate >40/min (1-5 years) *or* >30/min (>5 years)	SpO_2 <92% PEF <33% best or predicted • Exhaustion • Hypotension • Cyanosis • Silent chest • Poor respiratory effort • Confusion

Criteria for admission

- ✓ Increase β_2 agonist dose by giving one puff every 30–60 seconds, according to response, up to a maximum of ten puffs
- ✓ Parents/carers of children with an acute asthma attack at home and symptoms not controlled by up to 10 puffs of salbutamol via a pMDI and spacer, should seek urgent medical attention.
- ✓ If symptoms are severe additional doses of bronchodilator should be given as needed whilst awaiting medical attention.
- ✓ Paramedics attending to children with an acute asthma attack should administer nebulised salbutamol, using a nebuliser driven by oxygen if symptoms are severe, whilst transferring the child to the emergency department.
- ✓ Children with severe or life-threatening asthma should be transferred to hospital urgently.
- B **Consider intensive inpatient treatment of children with SpO_2 <92% in air after initial bronchodilator treatment.**

The following clinical signs should be recorded:

- Pulse rate – increasing tachycardia generally denotes worsening asthma; a fall in heart rate in life-threatening asthma is a preterminal event
- Respiratory rate and degree of breathlessness – ie too breathless to complete sentences in one breath or to feed
- Use of accessory muscles of respiration – best noted by palpation of neck muscles
- Amount of wheezing – which might become biphasic or less apparent with increasing airways obstruction
- Degree of agitation and conscious level – always give calm reassurance

NB Clinical signs correlate poorly with the severity of airways obstruction. Some children with acute severe asthma do not appear distressed.

Figure 1.6 - SIGN/BTS Guidelines for the management of acute asthma in children aged over 1 year[134].

1.4.3 Severity scores used to assess preschool wheeze

The severity of preschool wheeze can vary dramatically from mild to life-threatening[24, 135]. To ensure appropriate treatment, it is essential that the severity of exacerbations is assessed accurately[136]. Severity scores are simple yet effective tools for rapidly assessing the severity of a condition upon presentation, as well as the response to treatment, using serial measurements[137, 138]. Moreover, severity scores can be used to compare outcomes within and between hospitals for research[139].

Severity scores can also be useful for helping to decide management, such as whether to admit patients. These standardised tools can be especially useful to guide non-specialist clinicians, such as junior doctors, in clinical decision making. Additionally, severity scores can be useful for the prediction of specific end points, such as admission to ITU or discharge. Severity scores can also be useful when comparing the populations of different studies, to allow standardisation according to severity.

The 2017 NICE guidelines 'Viral-induced wheeze/infective exacerbation of asthma'[133], are a concise evidence-based guide for clinicians. These guidelines classify the severity of each child's exacerbation and help clinicians make management decisions[133]. However, guidelines are limited as they do not state the relative importance of each clinical observation within a category. Severity scores can often be more useful as they allow a more nuanced measurement of severity than a category alone[139].

A score specific to the preschool age is needed as some signs used in older children, such as inspiratory to expiratory (I:E) ratio[140, 141], can be difficult to measure in young children, due to the greater respiratory rate[142]. Additionally, severity scores for respiratory compromise in young children are often reliant on clinical observations, because pulmonary function tests can be unfeasible in preschool children with acute wheeze[139].

1.5 Management of preschool wheeze

1.5.1 Management of acute preschool wheeze

The NICE guidelines also provide a useful summary of the available evidence for the management of acute preschool wheeze[133]. In young children, inhaled short-acting beta agonists (SABAs), such as salbutamol, are recommended to provide

rapid symptomatic relief for wheezing exacerbations[11, 133]. The NICE guidelines recommend nebulised SABA for severe or life-threatening exacerbations, whilst metered-dose inhalers may be more appropriate in moderate exacerbations[133]. Nebuliser use in preschool wheeze should be avoided, as the efficacy of other modes of delivery, such as metered dose inhaler with spacer, are comparable[3]. The ERS recommended in 2014 that acute preschool wheeze can be treated with inhaled bronchodilators only, unless severe requiring oxygen, when nebulisers can be used[3, 7].

Controlled oxygen therapy is also recommended for all children with hypoxia, with the aim of maintaining saturations in the range 94-98%[133]. Antibiotics, such as amoxicillin, should only be prescribed if the presentation suggests bacterial infection[133]. The NICE guidelines recommend that if the patient has a previous asthma diagnosis, or asthma is strongly suspected, a short course of oral corticosteroids may be administered[133].

The acute management of EVW is very similar to the acute management of asthma in preschool children, except that there is no good evidence to endorse the use of oral corticosteroids (OCS) in EVW[131, 132]. An RCT by Oommen *et al.*, studying over 200 children aged 1-5 years, showed no significant difference between the placebo and intervention group, in terms symptom scores or hospital admission rates[132]. The intervention studied was 20 mg prednisolone once daily for five days, initiated by a parent[132]**.** In addition, a study of 700 preschool children who presented to hospitals in England showed that, for children with mild or moderate acute wheeze, there was no significant difference in the study outcomes when comparing oral prednisolone to placebo[131]. Based on current evidence, it is suggested that children treated in primary care should not

be given oral prednisolone, and that most children hospitalised children will not benefit from its use[3]. However further research into the use of OCS in very severe acute preschool wheeze is needed[3].

There is continued research exploring other potential treatment options for acute wheezing episodes in preschool children. Some evidence has shown that the use of azithromycin, a macrolide antibiotic, can significantly reduce the length of respiratory symptoms if started at their onset[143]. However, a Canadian study of 300 wheezing children aged 1-5 years, who presented to the Emergency Department, found no significant effect on the length of symptoms or interval before subsequent exacerbation[144]. There is currently no guidance about how this medication should be used and which patient groups should be targeted[23].

Another therapy to have been studied is 5% hypertonic saline[145]. A small pilot RCT of 41 preschool children suggested that hypertonic saline use was related to reduced length of stay and hospital admission rate among children presenting with acute wheeze[145]. These results require confirmation in a larger definitive trial.

The Alder Hey Children's Hospital (AHCH) guidelines for acute preschool wheeze are summarised in **table 1.2**. These guidelines provide a brief overview of the management, investigations and follow-up undertaken for any preschool child presenting with acute wheeze in Liverpool.

Treatment	Guidance
Oral steroids	Oral steroids are not recommended for children with acute preschool wheeze, although there is appreciation that some children who have a more asthma type picture will respond to this medication.
Inhaled salbutamol	Not all children with preschool wheeze will require a trial of salbutamol. Inhalers with spacers are recommended for children who are not hypoxic. Nebulised treatment is recommended for children who are hypoxic and require oxygen.
Use of high flow oxygen	This is a treatment that can be used for the treatment of hypoxia when unable to manage hypoxia on first line standard treatment (low flow nasal cannula). High flow oxygenation is not a treatment for increased or severe respiratory effort.
Investigations in the acute exacerbation	Blood gases are not routinely recommended as an investigation for preschool wheeze. Chest x-rays are not routinely performed on children with preschool wheeze.
Discharge	Ensure all children have an action plan and have had their inhaler technique checked.

Table 1.2 – AHCH Guidelines for the management of acute exacerbations of preschool wheeze

1.5.2 Management of recurrent preschool wheeze

There are currently no NICE guidelines specifically for preschool wheeze, however the asthma guidelines include specific guidance for children aged under 5 years[146]. In any child aged less than 5 years with suspected asthma it is recommended that

a SABA should be prescribed, to be used as a reliever, in combination with preventative treatments[146].

In 2014, the BMJ published a clinical review by Bush *et al.* of the available evidence for the treatment of preschool wheeze, including both the EVW and MTW phenotypes[3], which provides a valuable summary for clinicians. The current guidelines do not differentiate between MTW and EVW, with ICS used as the standard maintenance therapy in all preschool children with recurrent wheeze causing regular or troublesome symptoms[7, 26, 146]. When ICS therapy is chosen, it should be used on a trial basis, with a break used to see if treatment can be discontinued[2]. Furthermore, NICE guidelines recommend the use of montelukast in children aged less than 5 years with suspected asthma that is not controlled using regular low dose ICS[146].

It must be remembered that not all children require maintenance therapy, as children with mild symptoms may not benefit, and episodic symptoms require only intermittent therapy[3]. There is currently no convincing evidence that regular ICS use is beneficial to preschool children without interval symptoms between wheezing episodes[3]. Although, it has been highlighted that response rates may vary according to sex or ethnic group, meaning that further studies are required[147]. Intermittent use of ICS can reduce the risk of severe viral-induced wheezing episodes but can be associated with significant side effects in children, including growth suppression[29].

Whilst ICS are regarded as the first line maintenance treatment in preschool wheeze, there is some variation in practice, with some children with severe EVW offered montelukast, a leukotriene antagonist[26]. This practice is supported by no convincing evidence from RCTs in terms of reducing frequency or severity of

wheezing episodes[148]. As a result, a 2018 clinical review made the recommendation that montelukast should not be used, either regularly or intermittently, in preschool children with EVW[148]. Furthermore, montelukast can have significant adverse drug reactions, such as sleep disturbance and irritability[149]. On the other hand, it has been suggested that intermittent montelukast treatment can have a significant impact on the symptoms of acute respiratory tract infection in children with a positive modified Asthma Predictive Index (API)[110, 150].

Another potential treatment option, which is not yet widely used, is bacterial lysate therapy. A recent systematic review with meta-analysis showed that bacterial lysates decrease the frequency of preschool wheeze and asthma exacerbations in children[151]. The authors suggested that bacterial lysate therapy should be considered as an adjunct for the prevention of exacerbations of preschool wheeze and asthma[151]. Meanwhile, there remains a need for robust RCTs to determine the efficacy of this therapeutic strategy[151].

As in any chronic disease, the environmental exposures of each patient should be considered. This is illustrated by the risk of exacerbations associated with cigarette smoke exposure[2, 152]. A widely cited systematic review by Strachan *et al.* showed that there is a significantly increased risk of acute lower respiratory illness in children aged less than 3 years with cigarette smoke exposure, especially in cases of maternal smoking[152]. This emphasises the importance of encouraging smoking cessation in the families of children with wheeze.

Another important element of chronic disease management is patient education. There have been multiple studies investigating the impact of patient and caregiver education programmes on patient outcomes[2]. A trial of a multidisciplinary

education programme for the parents of children with asthma aged 0-4 years found that, following the programme, parents had improved knowledge and increased self-efficacy[153]. The intervention group was also found to have reduced healthcare use in the 12 months of follow-up[153]. These trends are supported by the findings of another interventional study, which concluded that a programme of education sessions, for caregivers of children aged less than 7 years with asthma, can improve the management of asthma and clinical outcomes of their children[154]. Conversely, a partially-blinded RCT of 200 children aged 18 months – 5 years, with acute wheeze, showed that there were no statistically significant differences in the main outcomes in the 12 month follow-up following a combination of educational interventions[155]. Some postulated reasons for the lack of benefit from the intervention included the shortage of age-appropriate advice, unstandardised treatment and a potentially inadequate intervention[155].The discrepancies in these findings emphasise the need for further research into the efficacy of preschool wheeze treatments, especially in relation to educational interventions.

1.5.3 Adherence to treatment in preschool wheeze

Suboptimal adherence to medication regimens is a universal challenge, but there are some additional challenges in relation to the preschool age group. For example, preschool children often have limited communication skills, meaning that they may not be able to accurately describe their symptoms and therefore may not receive appropriate therapy[156]. Also, a practical barrier to the administration of acute or preventative treatment in young children can be the difficulty in persuading them to stay sufficiently still to use a large volume spacer. Additionally, many young children are reliant on multiple caregivers, such as grandparents and childcare providers, thus unless all caregivers have satisfactory

understanding of wheeze and its treatment, adherence to treatment may be suboptimal[156]. Another factor limiting adherence to therapy is parental anxiety about the side effects of the medications, such as reduced growth velocity[29, 156]. It is vital to ensure that children and their carers understand how to administer each medication and appreciate the safety profile[23].

1.6 Prognosis of preschool wheeze

Multiple epidemiological studies have shown that preschool wheeze often remits by school age[1]. In the Tucson Children's Respiratory Study, nearly 60% of children who wheezed before their 3rd birthday no longer wheezed by 6 years[12]. However, Belgrave *et al.* found that children with atopy, persistent wheeze and frequent episodes are at risk of a gradual deterioration in lung function from early childhood until adolescence[157]. A recent meta-analysis has suggested that this reduction in lung function may persist into adulthood[158].

Consequently, it may be useful to categorise children with preschool wheeze according to the presence of atopic symptoms. Distinguishing patients with and without atopy can be beneficial since sensitivity to allergens in early childhood can be linked to higher risk of persistent symptoms and reduced lung function in later childhood[3, 24, 159]. Moreover, childhood wheezing with associated atopy has been linked to increased risk of subsequent Chronic Obstructive Pulmonary Disease (COPD)[158]. There have been trials exploring preventative therapies for asthma for those prone to early wheezing, including corticosteroids, but none of these strategies have yet been found to be effective in changing the natural history[3, 160].

It is currently impossible to distinguish between those children with transient wheeze and those children who will develop asthma, based only on their

presentation[161]. Additionally, no single biomarkers or genetic variants have been decisively linked to asthma development[123, 161]. Eosinophilic inflammation is commonly associated with asthma and recurrent wheezing, therefore blood eosinophils are potentially a strong candidate as a biomarker[162]. It has been shown that lack of eosinophilia in wheezy infant is predictive of future wheezing remission in most cases[163]. However, it should be noted that whilst eosinophil counts are often significantly raised in stable wheeze, this may not be true in acute wheeze[164]. Other candidate biomarkers include fractional exhaled nitric oxide (FENO), blood eosinophil-derived neurotoxin (EDN) and exhaled breath condensate (EBC) pH and cytokine content[162]. These biomarkers require further validation and some require adaptation to allow them to be more easily measured in preschool children[162].

Unfortunately, analysis of the inflammatory pathways in the lung which underlie preschool wheeze is limited by the fact that bronchoscopies are rarely performed in young children[165]. However, other methods have been employed to study these processes. These include urinary eosinophil protein X, which has been used to show that systemic activation of eosinophils occurs in viral-induced wheeze[166]. In another study, serum L-selectin was used as a marker of neutrophil activation, demonstrating that systemic neutrophil activation occurs in EVW[167].

The Asthma Predictive Index was produced from the Tucson cohort study[161]. It uses risk factors, such as eczema in the first three years of life, to predict the risk of wheeze persisting to school age[161]. Another notable birth cohort study performed in Leicester agreed with the Tucson original findings, but showed that the positive predictive value of the API is low[168]. On the other hand, it has a relatively high negative predictive value, so it can be used for identifying children

who are unlikely to develop asthma[161]. For example, in the Tucson cohort study, less than 3% of the children with a negative API at age 3 years had asthma during their school years[161, 169]. Another interesting consideration is the impact of the viral agent on the risk of asthma development. For instance, Jackson *et al.* found that when outpatient respiratory infections were analysed, wheezing associated with HRV infection carried a higher risk of subsequent asthma diagnosis than wheezing illness due to RSV[40]. More recently, novel machine learning approaches have been adopted to predict asthma[170]. This methodology has great potential, although existing studies have significant limitations, including insufficient sample size and unclear use of definitions[170].

1.7 Aims and objectives

The subject areas and chapters contained within this thesis are varied because of the constraints and uncertainties brought about by the COVID-19 pandemic. The content of this thesis does not attempt to address a single hypothesis but rather several objectives. However, all chapters relate directly to pre-school wheeze and asthma.

The specific objectives addressed by the work presented in this thesis include:

- To perform a systematic review of available severity assessment tools for preschool wheezing illness and to assess their validity for use
- To investigate expression of rhinovirus receptors (ICAM-1 and CDHR3) in respiratory epithelium in response to rhinovirus infection
- To characterise the demographic, clinical and social (including deprivation and pollution) characteristics of children hospitalised with viral induced wheeze/asthma in Liverpool

Chapter 2: Severity scores published for clinical use in acute wheeze in preschool children and their validation for use: a systematic review

2.1 Introduction

There have been two previous literature reviews of acute asthma severity scores used in preschool children, with the most recent published in 2004[138, 142]. These reviews were specific to preschool asthma, and since their publication more severity scores have been developed[136]. Furthermore, a literature review is a less rigorous scientific method than a systematic review, thus the findings are less meaningful[171]. Bekhof *et al*. undertook a systematic review, published in 2014, of paediatric dyspnoea scores, but this was not specific to the preschool child[136].

A contemporary systematic review relevant to acute preschool wheezing illness (including asthma) would allow those severity scores published for use in children aged 1-5 years to be identified and their measurement properties to be assessed[172]. Improving assessment, and thereby management, of preschool wheeze is vital as this age group has excessive ill-health and healthcare needs compared to older children with asthma[165].

The review question for this systematic review was:

What severity scores have been published for clinical use in acute wheeze in preschool children and how well have these been validated for use?

2.2 Methods

2.2.1 Study design

A systematic review of the severity scores published for use in acute wheeze in preschool children (age 1-5 years) was undertaken. This systematic review was registered with PROSPERO (registration ID CRD42020212507). The full protocol submitted can be found in **Appendix 1**. PROSPERO is an international database of

systematic reviews, with outcomes relevant to health, where protocols are registered prospectively[173]. PROSPERO aims to reduce duplication of work and prevent bias in the reporting of reviews[173]. This systematic review was undertaken in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA checklist in **Appendix 2**)[174].

2.2.2 Search strategy

Reviewer EW was responsible for the development of the search strategy. Moreover, EW completed the searches for each database and imported all results into citation management software. MEDLINE, Scopus, Web of Science and CINAHL databases were searched up to 30th November 2020, with no date or language restrictions.

MEDLINE provides access to a wide range of publications in the life science domain, with a particular focus on biomedical sciences[175]. The coverage starts from 1966, consists of over 5200 journals[175], and provides access to over 27 million references[175]. Scopus is an even larger database, with over 77 million items from over 5000 publishers[176]. Scopus is home to publications from a wide range of fields, including medicine, science, technology and humanities[176]. Over two thirds of the publications accessible via Scopus have been published since 1995, but cited references since 1970 are available[176]. Web of Science is another high-quality database, which serves a range of scientific disciplines[177].Web of Science has over 171 million records with coverage from 1900[177]. CINAHL is a prominent database for publications in nursing and other allied health professions[178]. It provides access to over 7.4 million records, from 290 journals[178]. CINAHL has considerable coverage, with publications available from 1963 onwards[178].

The references of all full-text papers eligible for inclusion were screened for relevant publications using backward searching. The same search terms were used for each database, grouped under the key words ‘wheeze’, ‘preschool’, ‘severity score’ and ‘acute’. Boolean operators were used to group terms, with ‘AND’ used between the groups of key terms and ‘OR’ used between synonyms in the same group. Truncation of some terms was used to account for different possible endings, to avoid inappropriate exclusion. The full list of search terms is available in **Appendix 3**. All study designs were included.

2.2.3 Inclusion and exclusion criteria and outcomes

Population	Children aged 1-5 who present acutely with wheezing illness to a healthcare setting.
Intervention	Scores that include at least two different parameters and have a numerical value assigned to each parameter. Scores eligible for inclusion were those that were evaluative or discriminatory, used to assess severity of acute wheeze in a clinical setting, and applied by a medical professional.
Comparison	All other relevant severity scores identified and their associated validity data.
Outcome	The main outcome was novel or modified severity scores published for acute wheeze in preschool children. Secondary outcomes included domains and items included in each severity score, number of severity scores that included each parameter, weighting applied to different parameters, country of origin, age of child and clinical setting. Other secondary outcomes relate to the methodological quality of the study (see below). The sensitivity and specificity for each score in predicting the outcome it was assessed for (such as admission) is recorded if available.

Table 2.1 – Inclusion criteria guided by the Population Intervention Comparison Outcome (PICO) framework[179].

2.2.4 Methodological quality assessment of severity scores

The framework for the methodological quality assessment was inspired by that used in previous systematic reviews of paediatric severity scores[136, 138, 142, 180]. The following definitions were used:

Term	Definition
Basis for item selection	The source of items in the score, such as previous scores, clinical observations, theory, expert judgements, or parent opinions[138].
Construct validity	The degree to which the score relates to existing measures of wheeze severity[180].
Criterion validity	How well scores generated by an instrument correlate with a gold standard[180].
Face validity	A qualitative judgement of the quality of the score as a measurement of preschool wheeze[138].
Reproducibility	The extent to which repeated measurements in the same people provide similar answers[180].
Internal consistency	The extent to which all items measure the same characteristic[138].
Interrater agreement	The extent to which multiple observers independently obtain similar scores[138].
Responsiveness	The ability of a score to detect changes over time[180].
Suitability for preschool children	Does not use invasive techniques or measurements which may be difficult or unreliable in young children, such as pulsus paradoxus or inspiratory to expiratory ratio[136, 142].

Table 2.2 – Definitions used for methodological quality synthesis

2.2.5 Screening and selection of literature

There were two main stages in the screening process. Duplicates were removed in two stages: first using the automatic function on EndNote reference manager, and then in a second stage of manual screening by author name and title. Once duplicates were removed, the titles and abstracts of all the identified publications were independently screened, using the inclusion and exclusion criteria, by two researchers (EW and WB). Any disagreements were settled by consensus and, if required, mediation was undertaken by a third party (DH). Eligible publications underwent screening of the full text, using the same criteria. All publications eligible for full text screening were entered into a table, and any reasons for

exclusion recorded (**Appendix 4**). Any non-English language full texts were translated by individuals with fluency in the required language.

2.2.6 Data extraction and synthesis

One reviewer (EW) extracted the relevant data into a predetermined data extraction table, which was checked by the other reviewer (WB). Any queries about the data extraction were discussed with the review supervisor (DH). No meta-analysis was undertaken as this was not appropriate for the aims of this systematic review.

2.2.7 Assessment and reduction of bias

Bias in this systematic review was reduced by performing an extensive search and rigorously applying the inclusion and exclusion criteria. Using two independent reviewers also minimised selection bias. The risk of bias/overall quality assessment of the identified publications was performed using tools relevant to each study type (**Appendix 6-9**). The risk of bias was assessed at the level of the individual study. Thus, RCTs (n=36) were assessed using the Cochrane risk of bias tool version 2[181], see **Appendix 6**. Using this tool, each of five domains is assigned either 'low risk of bias', 'some concerns' or 'high risk of bias', which are then used to generate an overall judgement[181].

In addition, quality assessment of cohort studies (n=42) was undertaken using the Newcastle-Ottawa scale (NOS)[182], see **Appendix 7**. This scale comprises three domains (selection, comparability and outcome[183]), with the total score ranging from 0 to 9, where a score of 9 would suggest minimal risk of bias[182, 183]. For this review, previously used categories have been used to classify the risk of bias, with a total score of 7-9 indicating low risk of bias, 5-6 moderate risk and 0-4 high risk[184]. Cross-sectional studies (5) were appraised using a modified version of the

NOS[185]. The case series (1) identified was appraised using the JBI Critical Appraisal Checklist for Case Series[186], see **Appendix 8**. For the remaining three publications, the JBI Critical Appraisal Checklist for Text and Opinion Papers was used[187], see **Appendix 9**.

2.3 Results

2.3.1 Search results

During the literature search, a total of 1355 studies were identified from the selected databases, and an additional 70 studies obtained from supplementary sources. Once duplicate publications were identified, 886 articles were screened, with 128 of these articles eligible for full-text screening. Of the 128 articles remaining, 41 were excluded (**Figure 2.1**).

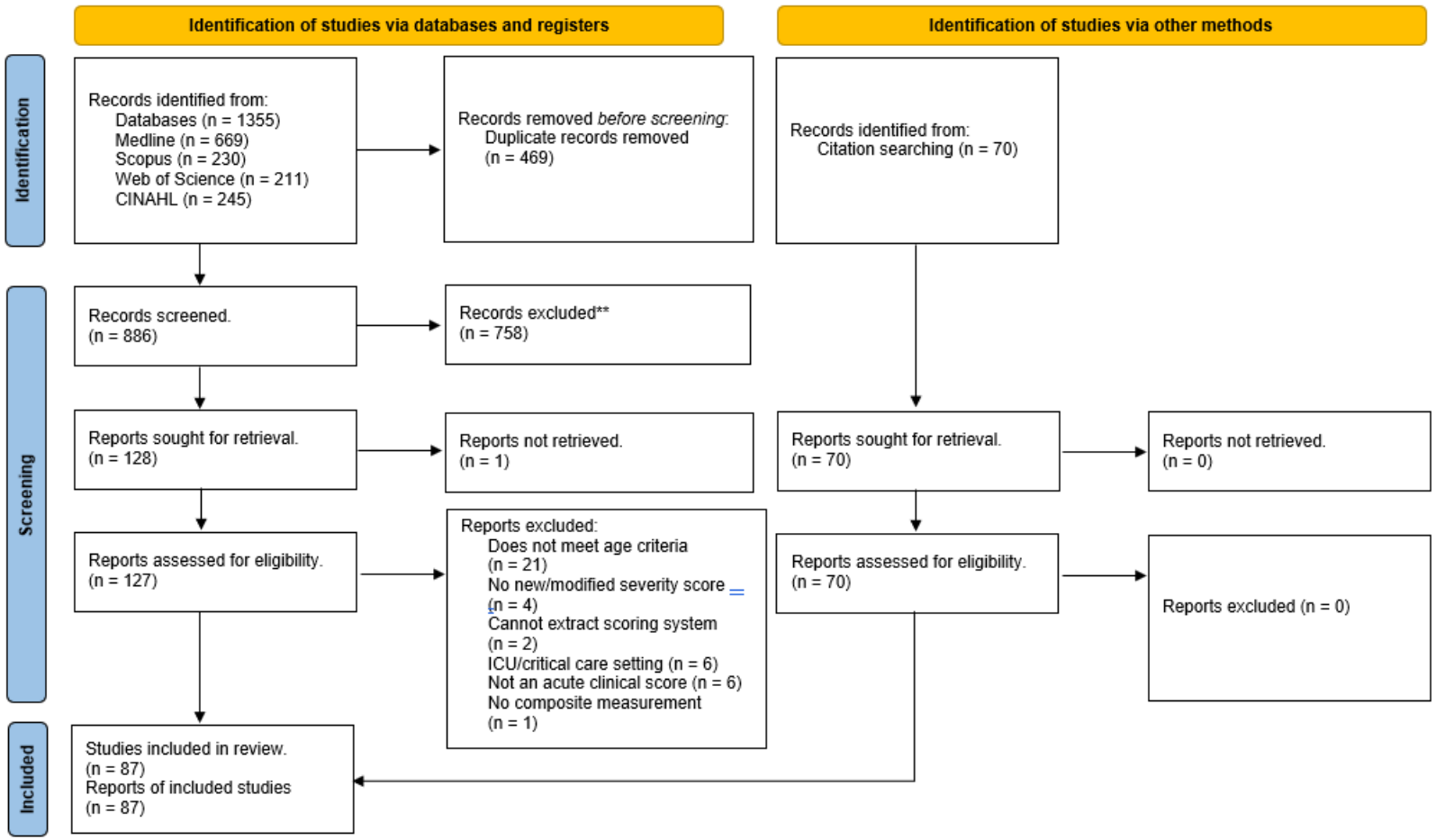


Figure 2.1 - PRISMA 2020 flow diagram for systematic reviews using databases and other sources, showing each stage of inclusion/exclusion[188]

In total, this systematic review identified 89 published scores which included preschool children (two publications presented two different scores). These were split into scores used only in the specified age group (6 months-6 years – n=5), scores developed using a sample including children aged over 5 years (n=29), scores developed using a sample including infants aged less than 1 year (n=44), and general paediatric measures (suitable for all children or an age range both above and below 6 months to 6 years) (n=11). The most common range of total scores was 0-12 points (n=21). Seventy severity scores had equal weighting of each domain. There were 38 modified severity scores and many were inspired by other scores, whilst some were based on theory, research, expert opinion or clinical observations (**Table 2.3**).

Author and Year	Basis for item selection	Age of participants
Alario *et al*. 1992[189]	Previous scores and theory	0-3 years
Ater *et al*. 2012[145]	Modified from a previous score	0-3 years
Bajaj *et al*. 2006[190]	Modified from a previous score	1-6 years
Bamberger *et al*. 2012[191]	Not stated	2-23 months
Bano *et al*. 2018[192]	Not stated	0-96 weeks
Bentur *et al*. 1992[193]	Modified from a previous score	1-6 years
Bentur *et al*. 1990[194]	Not stated	3 months - 2 years
Berger *et al*. 1998[195]	Derived from exisiting scores	5 months-2 years
Bierman *et al*. 1974[196]	Modified from a previous score	1-18 months
Bogie *et al*. 2007[197]	Modified from a previous score	Unspecified
Bohé *et al*. 2004[198]	Modified from a previous score	0-24 months
Can *et al*. 1998[199]	Derived from existing scores	7 weeks - 24
Caritg *et al*. 1999[200]	Not stated	0-2 years
Carroll *et al*. 2005[201]	Modified from previous score	
Chalut *et al*. 2000[202]	Previous scores and clinical	3-6 years
Chong *et al*. 2017[203]	Modified from a previous score	0-2 years
Coarasa *et al*. 2010[204]	Modified from a previous score	1 month-2 years
Coarasa *et al*. 2010[204]	Modified from a previous score	1 month-2 years
Connett *et al*. 1993[205]	Not stated	1.5-14.5 years
Constantopoulos *et al*. 2002[206]	Modified from a previous score	2 weeks-24 months
Conway *et al*. 1985[207]	Not stated	0.8-14.4 years
Dabbous *et al*. 1966[208]	Not stated	6 weeks-18 months
Dabbous *et al*. 1966[208]	Not stated	6 weeks-18 months
Daugbjerg *et al*. 1993[209]	Not stated	1.5-18 months
Davis *et al*. 1977[210]	Not stated	2-16 years
De Boeck *et al*. 1997[211]	Modified from a previous score	0-2 years
Deerojanawong *et al*. 1994[212]	Derived from existing scores	0-2 years
Devi *et al*. 1997[213]	Modified from a previous score	1-12 years
DiGiulio *et al*. 1993[214]	Modified from a previous score	2-16 years
Ducharme *et al*. 1997[215]	Not stated	2-17 years
Ejaz *et al*. 2015[216]	Not stated	1 month - 2 years
Freelander *et al*. 1984[217]	Previous research	3-13 years
Gajdos *et al*. 2009[218]	Not stated	0-15 months
Gern *et al*. 2002[219]	Not stated	0-18 months
Giordano *et al*. 2012[220]	Previous research	3-18 years
Giugno *et al*. 2004[221]	Modified from a previous score	0-24 months
Gorelick *et al*. 2004[222]	Expert opinion, previous scores and clinical findings	1-18 years
Groothuis *et al*. 1990[223]	Not stated	0- 24 months
Groothuis *et al*. 1993[224]	Modified from a previous score	0-36 months
Hambleton *et al*. 1979[225]	Modified from a previous score	1.5-7 years
Hurwitz *et al*. 1984[226]	Modified from a previous score	2-13 years
Hussein *et al*. 1986[227]	Modified from a previous score	0.8-14.7 years
Jartti *et al*. 2006[228]	Not stated	0-3 years
Kamps *et al*. 2014[229]	Derived from existing scores	2-18 years
Kelly *et al*. 2000[230]	Derived from existing scores	2- 18 years
Kerem *et al*. 1990[231]	Modified from a previous score	0.4-16 years
Kornberg *et al*. 1991[232]	Not stated	3-12 years
Kudukis *et al*. 1997[233]	Modified from a previous score	16 months-16 years
Lai *et al*. 2004[234]	Not stated	1-24 months
Levy *et al*. 2004[10]	Not stated	4-62 months
Liu *et al*. 2004[235]	Derived from previous scores	0-19 years
Lowell *et al*. 1987[236]	Theory, research and clinical practice	0-2 years
Macias *et al*. 2015[237]	Modified from a previous score	0-2 years

Magpuri *et al*. 2018[238]	Literature review and expert opinions	2-17 years
McCallum *et al*. 2013[239]	Modified from a previous score	0-2 years
McKenzie *et al*. 1979[240]	Modified from a previous score	0-16 years
Mejias *et al*. 2013[241]	Modified from a previous score	0-24 months
Moody *et al*. 2020[242]	Modified from a previous score	2-18 years
Needleman *et al*. 1995[243]	Not stated	2-18 years
Obata *et al*. 1992[244]	Modified from a previous score	0-5 years
Ochoa Sangrador *et al*. 2012[245]	Previous research and clinical finding	1-24 months
Pabon *et al*. 1994[246]	Modified from a previous score	4-12 years
Pancham *et al*. 2016[247]	Adapted from previous scores and theory	0-5 years
Parkin *et al*. 1996[248]	Theory and previous scores	1-5 years
Pavón *et al*. 1999[249]	Derived from previous scores	1-24 months
Pendergast *et al*. 1989[250]	Theory	3-6.8 years
Qureshi *et al*. 1998[251]	Modified from a previous score	2-18 years
Ralston *et al*. 2010[252]	Modified from a previous score	0-2 years
Reed *et al*. 2012[253]	Clinical observations and theory	0-2 years
Rivera *et al*. 2006[254]	Modified from a previous score	3-16 years
Rivera-Sepulveda *et al*. 2019[255]	Modified from a previous score	0-15 months
Rushton *et al*. 1982[256]	Not stated	2-17 years
Scarfone *et al*. 1993[257]	Modified from a previous score	1-17 years
Schuh *et al*. 1990[258]	Previous scores and studies	6 weeks-24 months
Singh *et al*. 1993[259]	Not stated	2-36 months
Singh *et al*. 1990[260]	Not stated	3-16 years
Singhi *et al*. 2014[261]	Modified from a previous score	1-12 years
Smith *et al*. 2002[262]	Modified from a previous score	5-17 years
Sritippayawan *et al*. 2000[263]	Derived from existing scores	4 months -4 years
Stevens *et al*. 2003[264]	Modified from a previous score	1 to 16 years
Tal *et al*. 1990[265]	Derived from existing scores	7-54 months
Uong *et al*. 2018[266]	Not stated (institutional score)	2-18 years
Vichyanond *et al*. 2013[267]	Derived from existing scores and clinical findings	1-12 years
Walsh *et al*. 2004[268]	Clinical observations	0.27-21.9 months
Wang *et al*. 1992[269]	Clinical experience and theory	0-2 years
Williams *et al*. 2011[270]	Derived from existing scores	0.6–8.27 years
Wishaupt *et al*. 2017[271]	Modified from a previous score	0-12 years
Wood *et al*. 1972[272]	Not stated	Not specified
Yung *et al*. 1996[273]	Modified from a previous score	0-19 years

Table 2.3 – List of severity scores and their basis for item selection.

Key: light blue = specific to 6 months-6 years, dark blue = general paediatric severity scores, yellow = developed in 1-5 years and above and purple = scores developed in 1-5 years and below.

Across the 89 scores, 24 different domains were identified. Domains were defined as overall groups of observations that the reviewers felt were similar or routinely collected together as part of a clinical assessment. These domains included 109 individual items (**Table 2.4** and **Appendix 5**). There were many different values for

respiratory rate used, often varying according to age (**Table 2.5**). Auscultation was commonly included (**Table 2.6**), being measured in 85/89 (96%) of the scores. However, several elements of wheeze were measured, including expiratory (59/89), particularly end expiratory (39/89), as well as inspiratory breath sounds/wheeze (57/89) and air entry/breath sounds (56/89). Wheeze audible without a stethoscope was also common, featuring in 32 of the scores. Accessory muscle use was measured in 85 scores (**Table 2.7**). Common subtypes of retraction were intercostal (32), subcostal (21), supraclavicular (17) and substernal (13). In addition, 23 scores assigned the total score a category, such as mild, moderate and severe.

The mean number of domains per score was 5.0 and the mean number of items per score was 9.5. Different parameters were used depending on the age of the children in the original sample (**Appendix 10**). For example, grunting was only found in severity scores developed in samples that included infants. Furthermore, nasal flaring was found in 47.7% of scores developed in infants and preschool school, but only 6.9% of scores developed using samples that included children aged over 5 years and excluded infants. Feeding or dehydration were far more commonly measured in scores developed in samples including infants, than in any other age group. On the other hand, pulsus paradoxus was not measured in any scores specific to preschool children or infants, being found in scores that were used for at least some children aged over 5 years. Dyspnoea or speech impairment were measured across all age groups, but in a significantly lower proportion of scores developed using samples that included infants (**Figure 2.2**). There were no signs identified that were specific to the 1-5 years age group.

Many severity scores included items difficult to measure in young children, such as I:E ratio (n=16) and pulsus paradoxus (n=3). Moreover, several scores could not be used by the whole healthcare team as they need specialist skills, such as identification of wheeze subtypes.

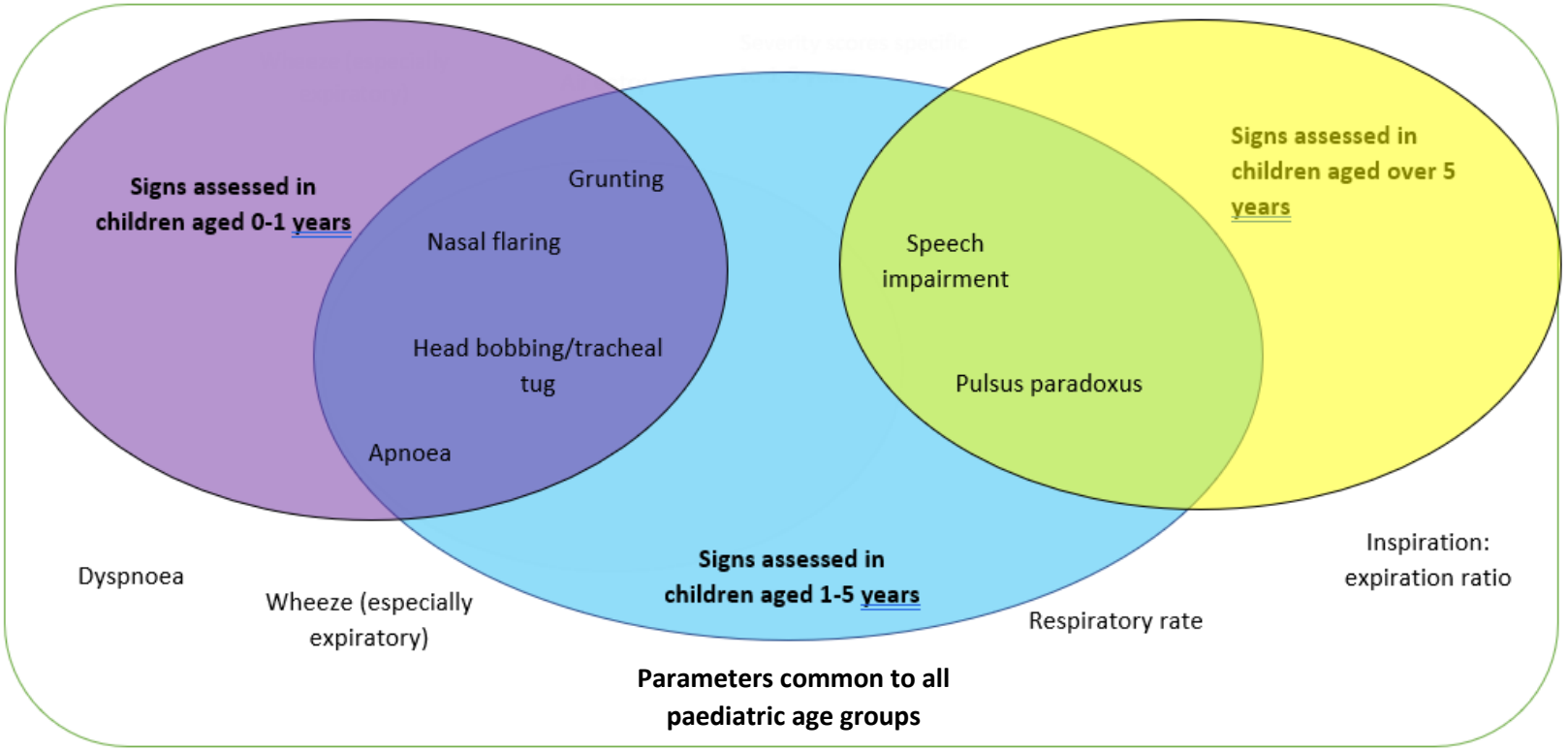


Figure 2.2 – A Venn diagram showing the overall trends in the parameters measured in scores designed for use in each age group, and how these relate to the parameters measured in the preschool age group.

Elements of sign measured	Alario *et al.* 1992	Arroyo *et al.* 2020	Bajaj *et al.* 2006	Bamberger *et al.* 2012	Bentur *et al.* 1990	Bentur *et al.* 1992	Berger *et al.* 1998	Bohé *et al.* 2004	Can *et al.* 1998	Caritg *et al.* 1999	Chong *et al.* 2017	Coarasa *et al.* 2010	Coarasa *et al.* 2010	Constantopoulos *et al.* 2002	Dabbous *et al.* 1966	Dabbous *et al.* 1966	Daugbjerg *et al.* 1993	De Boeck *et al.* 1997	Deerojanawong *et al.* 1994	Ejaz *et al.* 2015	Gajdos *et al.* 2009	Gern *et al.* 2002	Giugno *et al.* 2004	Groothuis *et al.* 1993	Groothuis *et al.* 1990	Jartti *et al.* 2006	Lai *et al.* 2004	Levy *et al.* 2004	Lowell *et al.* 1987
Respiratory rate			■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■		■	■	
O2 saturation		■		■						■				■				■					■	■	■			■	
Supplemental oxygen/ventilation										■														■	■				
Auscultation	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Audible wheeze	■				■		■		■			■	■	■	■	■		■			■		■			■			
Grunting	■			■											■	■													
Apnoea																						■	■						
Accessory muscle use/retractions	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■		■	■	■	■		■
I:E ratio															■	■	■									■			
Dyspnoea/speech impairment						■														■						■			
Nasal flaring	■			■	■		■	■	■	■	■	■	■		■	■			■	■	■					■			
Cyanosis/colour	■								■				■	■	■							■					■		
Mental status/consciousness/activity	■										■				■				■	■									
Heart rate					■	■		■		■		■		■														■	
Pulsus paradoxus																													
Cough/hoarseness				■											■							■							
Fever																						■							
Rhinorrhoea/secretions				■																		■							
Feeding/dehydration														■						■									
Resonance/hyperinflation															■														
Liver and spleen															■	■													
Age or weight of child																													
Duration of illness																						■							
Overall severity																	■											■	

Table 2.4 – Summary of parameters used in severity scores.

Key: purple = scores developed in the preschool age group (1-5 years) and below, light blue = preschool age specific (6 months-6 years), yellow = scores for preschool age group and above and dark blue = general paediatric scores

Elements of sign measured	Macias *et al.* 2015	McCallum *et al.* 2013	Mejias *et al.* 2013	Obata *et al.* 1992	Ochoa Sangrador *et al.* 2012	Pancham *et al.* 2016	Pavón *et al.* 1999	Ralston *et al.* 2010	Reed *et al.* 2012	Rivera-Sepulveda *et al.* 2021	Schuh *et al.* 1990	Singh *et al.* 1990	Walsh *et al.* 2004	Wang *et al.* 1992	Ater *et al.* 2012	Bano *et al.* 2018	Chalut *et al.* 2000	Parkin *et al.* 1996	Tal *et al.* 1990	Connett *et al.* 1993	Davis *et al.* 1977	Dev *et al.* i 1997	DiGiulio *et al.* 1993	Ducharme *et al.* 1997 1997	Freelander *et al.* 1984	Giordano *et al.* 2012	Gorelick *et al.* 2004	Hambleton *et al.* 1979	Hurwitz *et al.* 1984	Kamps *et al.* 2014
Respiratory rate	■	■	■	■		■	■	■		■		■		■	■	■		■	■	■		■	■		■	■				■
O2 saturation		■	■						■	■					■	■	■													■
Supplemental oxygen/ventilation						■																								■
Auscultation	■	■	■	■	■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Audible wheeze		■			■		■				■	■		■			■		■					■		■				
Grunting																														
Apnoea																														
Accessory muscle use/retractions	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
I:E ratio																		■	■				■				■			
Dyspnoea/speech impairment					■										■	■		■		■		■			■					■
Nasal flaring										■	■	■		■							■									
Cyanosis/colour					■		■					■																		
Mental status/consciousness/activity			■	■	■									■						■									■	■
Heart rate													■												■					
Pulsus paradoxus																									■					
Cough/hoarseness																														
Fever																														
Rhinorrhoea/secretions																														
Feeding/dehydration			■						■			■	■	■																■
Resonance/hyperinflation																				■										
Liver and spleen																														
Age or weight of child									■				■																	
Duration of illness																														
Overall severity														■										■						

Table 2.4 continued – Summary of parameters used in severity scores.

Key: purple = scores developed in the preschool age group (1-5 years) and below, light blue = preschool age specific (6 months-6 years), yellow = scores for preschool age group and above and dark blue = general paediatric scores

Elements of sign measured	Kelly *et al.* 2000	Kornberg *et al.* 1991	Kudukis *et al.* 1997	Magpuri *et al.* 2018	Moody *et al.* 2020	Needleman *et al.* 1995	Pabon *et al.* 1994	Pendergast *et al.* 1989	Qureshi *et al.* 1998	Rivera *et al.* 2006	Rushton *et al.* 1982	Scarfone *et al.* 1993	Singh *et al.* 1990	Singhi *et al.* 2014	Smith *et al.* 2002	Stevens *et al.* 2003	Uong *et al.* 2018	Vichyanond *et al.* 2013	Bierman *et al.* 1974	Carroll *et al.* 2005	Conway *et al.* 1985	Hussein *et al.* 1986	Kerem *et al.* 1990	Liu *et al.* 2004	McKenzie *et al.* 1979	Williams *et al.* 2011	Wishaupt *et al.* 2017	Wood *et al.* 1972	Yung *et al.* 1996
Respiratory rate	■				■	■		■	■			■		■	■	■	■	■	■	■		■	■	■		■	■		
O2 saturation	■		■	■	■		■		■	■		■	■	■			■	■		■						■	■		
Supplemental oxygen/ventilation	■		■		■		■		■																			■	
Auscultation	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Audible wheeze				■								■	■		■				■		■				■				■
I:E ratio																													
Grunting																											■		
Apnoea	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■
Accessory muscle use/retractions				■								■				■			■	■									
Dyspnoea/speech impairment	■							■	■					■		■		■				■	■	■		■			
Nasal flaring						■																							
Cyanosis/colour											■		■									■						■	
Mental status/consciousness/activity			■				■			■												■						■	
Heart rate								■												■	■		■						
Pulsus paradoxus								■															■						
Cough/hoarseness																											■		
Fever																											■		
Rhinorrhoea/secretions																											■		
Feeding/dehydration																													
Resonance/hyperinflation																									■				
Liver and spleen																													
Age or weight of child																													
Duration of illness																											■		
Overall severity						■																							

Table 2.4 continued – Summary of parameters used in severity scores.

Key: purple = scores developed in the preschool age group (1-5 years) and below, light blue = preschool age specific (6 months-6 years), yellow = scores for preschool age group and above and dark blue = general paediatric scores.

Age group	Normal value (breaths per minute)	Low severity values (breaths per minute)	Medium severity values (breaths per minute)	High severity values (breaths per minute)	Frequency
1-2 years	-	≤40	41-44	≥45	5
	≤30	31-45	46-60	≥60	1
	20-40	-	41-55	>55	1
	-	≤35	35-50	>50	1
	<30	30-39	40-50	>50	1
	-	20-40	41-60	>60	1
	≤30	31-45	-	>45	1
2-3 years	-	≤34	35-39	≥40	6
	-	<35	35-39	>39	1
	18-26	27-34	35-39	≥40	1
	≤26	27-34	35-39	≥40	1
1-3 years	<25	25-34	35-44	≥45	1
4-5 years	-	≤30	31-35	≥36	7
	16-24	25-30	31-35	≥36	1
	≤24	25-30	31-35	≥36	1
3-6 years	<20	20-24	25-34	≥35	1

Table 2.5 – Age-specific respiratory rates in the included severity scores and frequency of use.

NB: Only scores with values specific to a small age bracket are presented. Many scores used the same categories for all children or only two age categories, such as above and below 6 years.

Auscultation domain	Item descriptors
Wheeze severity (with stethoscope)	No wheeze Wheezing doubtful Mild wheeze Moderate wheeze Severe/marked wheeze
Expiratory wheeze (with stethoscope)	None End expiratory wheezing only Expiratory wheeze (1/2) Expiratory wheeze (3/4) Wheeze throughout expiration (1)
Inspiratory wheeze (with stethoscope)	None Early inspiratory wheeze Part inspiratory wheeze Full inspiratory wheeze
Inspiratory breath sounds (with stethoscope)	Normal inspiratory breath sounds Unequal inspiratory breath sounds Decreased/absent inspiratory breath sounds
Audible wheeze (without stethoscope)	None Audible expiratory wheeze Audible inspiratory and expiratory wheeze
Rhonchi	None Scattered rhonchi Widespread/numerous rhonchi
Rales/crackles	None Mild rales Loud rales Decreased to absent rales Inspiratory crackles Expiratory crackles
Wheeze location	None Local/segmental wheeze (<4 fields) Diffuse wheeze (≥4 fields) Obvious wheeze all areas
Breath sounds	Normal breath sounds, Diminished breath sounds No breath sounds/silent chest
Air entry/air exchange/aeration	Good Fair Moderate decrease Obvious decrease Marked decrease/minimal/poor/barely audible
Decreased air entry localisation	None Localised decreased air entry Air entry decreased at bases Widespread/multiarea decrease in air entry
Air entry symmetry	Regular symmetrical air entry Asymmetrical air entry

Table 2.6 – Summary of the descriptors identified for the auscultation domain

Accessory muscle use/retraction domain	Item descriptors
General retractions/indrawing	None/absent Minimal/barely visible Marked/severe Maximal
Intercostal retraction location	None Inferior Superior Generalised
Intercostal retraction severity	None Minimal Moderate Marked/severe Extreme/maximal
Subcostal retraction	None Minimal Moderate Marked/severe Extreme/maximal
Substernal retraction	Absent Present
Supraclavicular retraction	None Mild Moderate Marked
Elevation of clavicle	Absent Present
Suprasternal retraction/ tracheal tug	Nil Present Pronounced
Head bobbing	Absent Present
Xiphoid retraction	None Minimal Marked
Lower costal retraction	Absent Present
Abdominal muscle use	Absent Present
Paradoxic breathing/thoracoabdominal paradox	Absent Present
Chest movement	Equal Respiratory lag Seesaw respiration
Neck strap/sternocleidomastoid use	Absent Present
Hyperinflation	Absent Present

Table 2.7 – Summary of the descriptors identified for the accessory muscle use/retractions domain

2.3.2 Setting

There were 42 severity scores for use in the emergency department or primary care, 36 scores for inpatient use and 4 for use in both settings. The most common countries for severity score development were the USA (36 scores), Canada (n=7), the UK (n=4), India (n=4), Australia (n=4) and Israel (n=4), see **Table 2.8**. The majority of publications identified were English language, however some Spanish[198, 200, 204] (n=3) and German[274] (n=1) publications were also eligible for inclusion.

Country	Frequency
Argentina	3
France	1
Australia	4
Spain	2
Israel	4
Chile	1
Germany	1
UK	4
USA	36
The Netherlands	2
Canada	7
Thailand	3
India	4
Puerto Rico	1
Japan	1
South Africa	0
Finland	1
Pakistan	1
Denmark	1
Taiwan	1
Brazil	1
Belgium	1
Turkey	1
Greece	1
Singapore	1
Ireland	2

Table 2.8 – The country of origin of each of the severity scores. Some scores had multiple countries involved in development or validation.

2.3.3 Methodological assessment of scores

The methodological quality assessment of the severity scores showed that many scores had no associated validity data and none of the scores measured all specified characteristics of

the score (see **Table 2.9**). The most commonly reported characteristic was the interobserver reliability, which was reported by 22 studies. Construct validity was the next most reported characteristic (15 studies), followed by reproducibility (7), face validity (5), discriminatory power (4), responsiveness (3), internal consistency (3) and criterion validity (2). The sensitivity and specificity for a specified outcome was reported in only 10 studies. Several different outcomes were predicted, including admission/hospitalisation, intensive care unit (ICU) admission, oxygen and intravenous treatment.

The three most validated severity scores identified in this review were the Clinical Asthma Score (CAS)[248], Pediatric Asthma Severity Score (PASS)[140] and Preschool Respiratory Assessment Measure (PRAM)[275, 276]. The predetermined criteria used to assess these scores can be found in the methods section[138, 180, 277, 278].

There was only one well-validated score developed specifically for use in children aged 1-5 years identified - the CAS[248]. The CAS was created for use in hospitalised children (Parkin *et al.*, 1996)[248]. This score was developed using two samples of children. Initially 28 children were recruited to guide item selection and to assess discriminatory power and interobserver reliability[248]. Next, 30 patients with preschool wheeze were selected to assess the responsiveness and validity of the score[248]. The final CAS includes five parameters, each scored from zero to two points. These are: respiratory rate, inspiration to expiration ratio, wheezing, observed dyspnoea and indrawing[248].

The development of this score was performed to a high standard, with a scientific basis for item selection and thorough examination of the measurement properties of the score. The interrater reliability for the score was high, for example the weighted kappa coefficient for agreement between the two physicians was 0.82[248]. In addition, the CAS was shown to have strong discriminatory power, with a Ferguson's δ value of 0.92[248]. The internal consistency of the score using this sample was good, with a Cronbach alpha for the final

score of 0.86[248]. The responsiveness of this severity score was satisfactory, with the difference in CAS between admission and discharge being significant when analysed using the Wilcoxon signed rank test ($p < 0.01$)[248]. A survey of five paediatricians found that the face validity of the five-item score was good[248]. Furthermore, the construct validity was shown to strong, with a Spearman's rank coefficient of 0.47 between length of stay and total CAS on admission ($p<0.05$)[248]. There was also a strong negative correlation (-0.58,) noted between the drug-dosing interval and CAS ($p < 0.01$)[248]. Moreover, this score is relatively easy to apply, with only a stethoscope needed to complete it[248]. More research is needed to assess this score in other settings, such as ED and outpatient clinics[248]. Parkin and colleagues suggested that this score could be used for guiding clinical decisions, such as fitness for discharge or drug dosing schedules, or as an outcome measure for clinical trials of preschool children with wheeze[248].

The PASS was validated by Gorelick and colleagues, in over 1200 children aged between 1 and 18 years with acute asthma attending the ED[140]. It should be noted that, unlike in the development of some other severity scores, this population included the full spectrum of wheeze severity[140]. Whilst three different composite measurements were evaluated, the final score comprises only three domains: work of breathing, wheezing and prolonged expiration, each scored from zero to two[140].

The interobserver reliability of the PASS was good, with a weighted Kappa value of 0.83 between pairs of like observers, such as two physicians[140]. The area under the ROC curve was calculated to determine discriminative power of the score[140]. This was shown to be above 0.8 for both emergency departments, for discriminating admission versus discharge as well as requiring and not requiring admission[140]. The responsiveness of the score was assessed using the percentage change of the total score from admission to disposition from the ED. The percentage change was significantly different in the discharged group (range:

51-79%) versus the group that was admitted (25-32%)[140]. The construct validity was also assessed by comparison with peak flow rate and oxygen saturation[140]. There was a modest but statistically significant correlation between the PASS and peak flow rate (Pearson's correlation coefficient = -0.22 at admission and -0.28 at discharge)[140]. There was also a significant correlation between the PASS and oxygen saturation (correlation coefficient = -0.42 at admission and -0.28 at discharge)[140]. The relative simplicity of the PASS is cited as a potential strength of this score over other scores with similar validity for use[140]. These results show that the PASS could be a useful clinical tool in the ED for helping to decide which patients to admit and discharge[140].

Another commonly cited score is the PRAM (Chalut *et al.*, 2000), for children aged 3-6 years[202]. The PRAM was developed for ED use, and the initial study had a sample size of 217 (n=145 for testing and n=72 for validation)[202]. The PRAM consists of five parameters: suprasternal retractions, scalene muscle contraction, air entry, wheezing and oxygen saturation[202]. The total score ranges from zero to twelve[202].

The PRAM was initially used in the test group and found to have modest discriminatory power ($r^2 = 0.16$, P = 0.001) and responsiveness ($r^2 = 0.13$, P = 0.05)[202]. The criterion validity was measured using predicted respiratory resistance and it was found that the Spearman's rank correlation between the PRAM and percentage change in respiratory resistance from baseline was 0.32 in the test group and 0.58 in the validation group[202]. The authors suggested that this tool can be used in the emergency department for documentation of acute severity of preschool wheeze and monitoring the response to treatment[202].

Reference	Reproducibility	Internal consistency	Interobserver agreement	Face validity	Criterion validity	Construct validity	Responsiveness	Discriminatory power	Sensitivity and specificity for predicting outcome
Alario *et al.* 1992[189]			■						
Ater *et al.* 2012[145]			■						
Bentur *et al.* 1992[193]			■						
Carroll *et al.* 2005[201]	■		■			■			Sensitivity and specificity to determine ICU admission
Chalut *et al.* 2000[202]	■			■	■		■	■	
Chong *et al.* 2017[203]									Statistically significant in predicting respiratory support, IV hydration, length of stay and admission
Coarasa *et al.* 2010[204]									Sensitivity and specificity to predict hypoxaemia
Connett *et al.* 1993[205]									Sensitivity and specificity for predicting the need for IV treatment
Dabbous *et al.* 1966[208]	■		■						
Gajdos *et al.* 2009[218]	■		■						
Gorelick *et al.* 2004[222]			■	■		■	■	■	Area under ROC curve to discriminate admitted vs discharge and admission vs not requiring admission
Groothuis *et al.* 1990[223]			■						
Hurwitz *et al.* 1984[226]						■			
Kamps *et al.* 2014[229]		■	■						Sensitivity and specificity calculated
Kerem *et al.* 1990[231]									Sensitivity and specificity for predicting hospitalisation
Levy *et al.* 2004[10]			■	■		■			
Liu *et al.* 2004[235]			■			■			

Reference	Reproducibility	Internal consistency	Interobserver agreement	Face validity	Criterion validity	Construct validity	Responsiveness	Discriminatory power	Sensitivity and specificity for predicting outcome
Lowell *et al.* 1987[236]			■			■			
Macias *et al.* 2015[237]						■			
Magpuri *et al.* 2018[238]				■					
McCallum *et al.* 2013[239]	■	■	■						Area under receiving operator curve for predicting oxygen
Needleman *et al.* 1995[243]									Mentions sensitivity for detecting changes in clinical status
Obata *et al.* 1992[244]	■					■			
Parkin *et al.* 1996[248]			■	■		■	■	■	
Pavón *et al.* 1999[249]						■			Sensitivity and specificity for detecting hypoxaemia
Qureshi *et al.* 1998[251]			■						
Reed *et al.* 2012[253]								■	Sensitivity and specificity for predicting mortality
Rivera-Sepulveda *et al.* 2021[255]		■	■						
Scarfone *et al.* 1993[257]			■						Hospitalised patients had a significantly higher median PI
Smith *et al.* 2002[262]			■		■	■			
Sritippayawan *et al.* 2000[263]						■			The sensitivity and specificity in predicting hypoxaemia
Stevens *et al.* 2003[34]			■						
Vichyanond *et al.* 2013[267]			■						Sensitivity and specificity for admission/discharge
Walsh *et al.* 2004[268]									The specificity and sensitivity for predicting admission
Wang *et al.* 1992[269]			■			■			
Wood *et al.* 1972[272]						■			
Yung *et al.* 1996[273]	■		■			■			

Table 2.9 – Methodological quality assessment of severity scores with available data.

2.3.4 Quality assessment and risk of bias

Risk of bias/quality assessment tools were used to allow comparison between different studies, and study designs, according to methodological rigour. Using the Cochrane Risk of Bias 2 tool[181], out of the 36 RCTs identified, only one study was found to have low risk of bias, 20 had some concerns and 13 had high risk of bias (**Appendix 6**). Many of the studies shared the same flaws, for instance, few studies carried out an intention-to -treat analysis to account for loss to follow-up, several had a high rate of drop out and only a small number pre-published their plans for data analysis. A total of 47 studies were evaluated using the Newcastle-Ottawa Scale[183], comprising 42 cohort studies and 5 cross-sectional studies (**Appendix 7**). Of the identified studies, 15 were deemed to have low risk of bias, 32 had moderate risk of bias, and none had high risk. Common flaws in the study design were lack of comparability between groups, often with no adjustment for age, and high loss to follow up. For the quality assessment of the remaining four studies see **Appendix 8 and 9**. Whilst the overall risk of bias of the eligible studies is reasonably high and the quality relatively low, this does not affect the quality of the severity score used in each study, which is the primary outcome of this systematic review.

Chapter 3: Investigating the effects of rhinovirus infection on the expression of rhinovirus receptors (ICAM-1 and CDHR3) and cytokine release from respiratory epithelial cells

3.1 Background

There are three known rhinovirus receptors, ICAM-1, LDLR and CDHR3[54]. ICAM-1 is the main receptor for HRV-A and B (the major group) and has been extensively studied, although its immunological role is still incompletely understood[54]. LDLR is the receptor for minor group HRV[53]. HRV-C was more recently discovered in 2006[53], and as such, there is less understanding of the immunological pathways concerning its receptor (CDHR3) and the epithelial response to HRV-C infection[53, 279].

There has been some research into the relationship between HRV-A and B infection and receptor expression in epithelial cells. The A549 cell line has been used to investigate ICAM-1 expression following HRV infection, using flow cytometry and PCR[74]. ICAM-1 surface expression was found to increase three-fold[74]. Additionally, a study using human nasal epithelial cells used immunocytochemistry to show that HRV infection increases surface expression of ICAM-1[280].

Evidence from cell culture experiments have shown that transmission of HRV-C between cells, during plaque formation, is reliant on the expression of CDHR3[279]. No studies using cell lines to successfully culture HRV were identified[281]. Meanwhile, a notable study by Griggs *et al.* showed that HRV-C infection reduces expression of CDHR3 in primary human bronchial epithelial cells, using multiple techniques including flow cytometry and immunofluorescence[282].

Interleukin-6 is a proinflammatory cytokine produced in response to inflammatory and infective stimuli by epithelial cells. It is commonly used as a marker of epithelial

inflammation and infection in cell biology. IL-6 plays a role in promoting differentiation of Th2 cells and inhibiting differentiation of Th1 cells[283]. It has previously been shown that IL-6 secretion is increased following HRV infection[87, 284, 285]. Subauste *et al.* used the BEAS-2B cell line to demonstrate that IL-6 secretion is increased following HRV-14 infection, using RT-PCR[87]. Moreover, Terajima *et al.* used primary tracheal epithelium to show that IL-6 release into supernatant is increased following infection with HRV-2 and HRV-14, by enzyme-linked immunosorbent assay (ELISA)[284].

The release of proinflammatory substances, including IL-6, is associated with the severity of symptoms during human respiratory tract infection[57]. There is no single generic inflammatory response to HRV infections, but a number of different virus-specific pathways of signalling, each induced once the individual strain of virus binds to its corresponding receptor[72]. Developing a better understanding of the inflammatory pathways, and how they relate to the HRV serotypes, may allow advancement of therapy targeted according to the receptor-binding behaviour of the HRV responsible for the infection[72].

The experiments within this chapter were undertaken to investigate the relationship between the expression of rhinovirus receptors on respiratory epithelium, and the epithelial inflammatory response following HRV infection.

3.1.1 Aims

The aims of this study were:

- To measure the baseline expression of ICAM-1 and CDHR3 receptors in A549 and BEAS-2B cells.
- To investigate how ICAM-1, CDHR3 and IL-6 expression vary in BEAS-2B cells infected with HRV (species A and C) and undertake a time-course analysis.
- To investigate whether HRV receptor expression correlates with the inflammatory response in HRV infected airway epithelial cells

3.2 Materials and methods

3.2.1 Reagents

Reagent	Supplier	Catalogue Reference
Acetic acid	Sigma	W200611
CDHR-3 probe	Applied Biosystems	4331182
Chloroform	Sigma	C2432
Dulbecco's Modified Eagle Medium (DMEM) with high glucose	Sigma	D6429
Ethanol	Fisher	E/0600DF/17
Foetal Bovine Serum (FBS)	Invitrogen	10270106
Gentamicin	Sigma	G1397
High-capacity cDNA reverse transcription kit	Applied Biosystems	4368814
ICAM-1 probe	Applied Biosystems	4331182
IL-6 probe	Applied Biosystems	4331182
Isopropanol	Sigma	24137
MRPL-32 probe	Applied Biosystems	4331182
Phosphate Buffered Saline (PBS)	Fisher	BP3994
qPCR Master Mix	PrecisionPLUS	PPLUS-R-XXML
Rat tail collagen type 1	Gibco	11519816
Trypan blue	Gibco	15250-61
Trypsin	Sigma	59427C

Table 3.1 Reagents used, along with the details of the supplier and the catalogue reference number

3.2.2 Respiratory cell lines

The A549 cell line is derived from a human adenocarcinoma of the lung[286]. It is a useful respiratory model as it shares biochemical and structural properties with type II cells of the pulmonary alveoli[286]. The BEAS-2B cell line of human bronchial epithelium, which has undergone adenoviral transformation[287, 288]. This cell line retains electron microscopic features of epithelial cells[287]. The BEAS-2B cell line is often preferred over A549 as it is not derived from cancer cells, which may have undergone changes in differentiation, thus altering their immunological responses to infection. Since BEAS-2Bs are derived from bronchial epithelium, it is often used to study asthmatic inflammation.

Preliminary experiments were undertaken in A549 cells. BEAS-2Bs were used for HRV infection. Originally, I had anticipated infecting primary nasal epithelial cells with HRV but because of time constraints due to COVID-19, I was unable to do this.

3.2.3 A549 cell culture

A549 alveolar epithelial type II-like cells were sourced from Sigma-Aldrich, Gillingham, UK. These cells were cultured in submerged conditions in DMEM with 10% FBS and 1% gentamicin. Cells were split twice weekly and stored in a humidified incubator at 37^{o}C with 5% carbon dioxide. The cells were grown in 75cm^{2} CELLSTAR cell culture flasks (Greiner Bio-One International, Frickenhausen, Germany). A549 cells used were not passaged more than 20 times.

For subculture, cells were detached using 3ml of 1x trypsin solution and returning the flask to the incubator for 3-5 minutes. The cells were visualised using the EVOS™ XL Core Imaging System (ThermoFisher Scientific, Massachusetts, USA) light microscope until at least 90% were trypsinised from the bottom of the flask. If this was not achieved, the flask was returned to the incubator for an extra minute to aid the dissociation of cells. Once this step was satisfied, 6ml of DMEM was added to the flask to neutralise the trypsin. Then all 9ml contained in the flask was placed into a 10ml tube and centrifuged for 5 minutes at 2000rpm. The centrifuge used for this step was the Centrifuge 5810R (Eppendorf AG, Hamburg, Germany). The supernatant was discarded, and the remaining cell pellet resuspended, by gently triturating 10 times, in 1ml of DMEM. A549 cells were seeded in T75 flasks at a density of 200,000 cells per flask, using an R1 cell counter (Olympus corporation, Tokyo, Japan) to guide the dilution.

Cells were utilised when approximately 70% confluent. Confluence was determined by eye using light microscopy. Over 90% of cells were consistently viable, determined using trypan blue exclusion. At least n=3 samples of each cell line were acquired for each run. A

microbiological safety class II cabinet was used for all steps where contamination of the cells was possible.

3.2.4 BEAS-2B cell culture

BEAS-2B cells were cultured in DMEM, with 10% FBS and 1% gentamicin. BEAS-2B cells were seeded onto a plate pre-coated with 0.03 mg/mL rat tail collagen type I. Before cell seeding, 75µl of collagen was added to the flask (along with 4.5ml of 20mM acetic acid) and left at room temperature for one hour, on a level surface, to allow sufficient coating. Following this, the collagen solution was aspirated, and the flask washed three times with 5ml of sterile PBS. The same process for cell seeding and culture was used as for A549 cells. BEAS-2B cells were not passaged more than 15 times. All experiments were replicated with at least three independent cell passages.

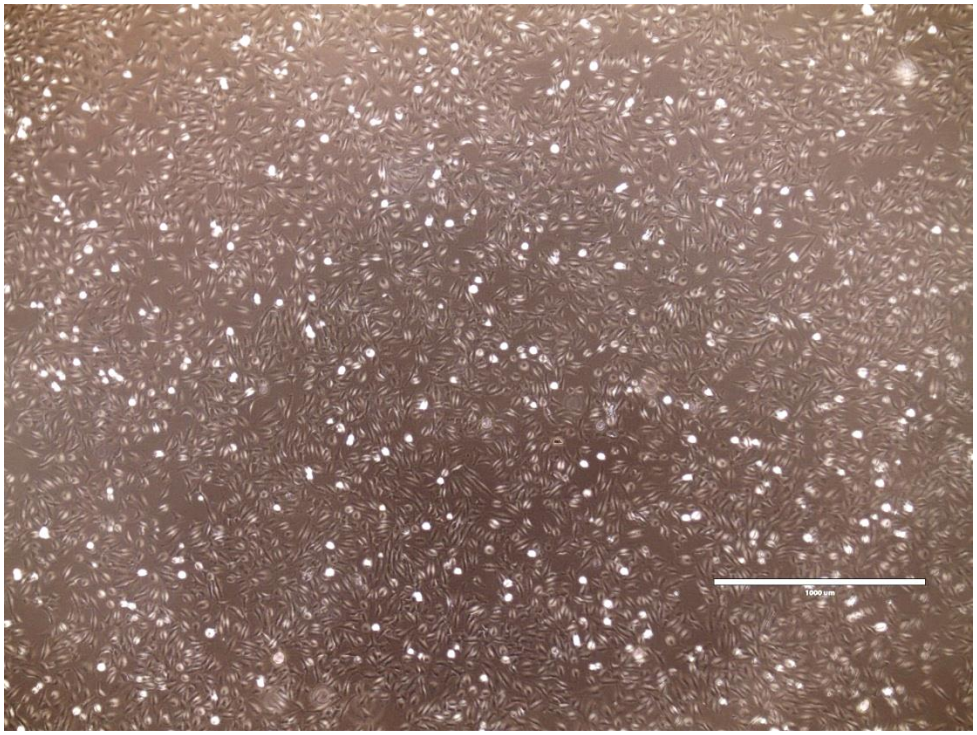


Figure 3.1 – Morphology of uninfected BEAS-2B (passage 12), x4 magnification.

3.2.5 HRV infection

BEAS2B cell cultures were infected with HRV species A and C at a multiplicity of infection (MOI) of 1. The calculation undertaken to find the correct multiplicity of infection was the number of plaque forming units (PFU) divided by the number of cells. The total volume of each well was made up to 1000µl, using cell culture media mentioned previously.

5 x 10^5 BEAS-2B cells were cultured in 12-well sterile cell culture plates (CellStar, Greiner Bio-One International) for 48 hours, when confluence was achieved. At this point, virus was added, and incubation at 37°C continued for several intervals between 30 minutes and 24 hours. A time course rhinovirus-induced expression of ICAM-1, CDHR3, IL-6 and L-32 mRNA was undertaken to assess the peak of infection, with measurements taken at these intervals: 30 minutes, 1 hour, 2 hours, 4 hours and 24 hours. These samples were compared to a control, obtained at time = 0 minutes.

3.2.6 RNA extraction

BEAS-2B cells were lysed using phenol and guanidine isothiocyanate reagent (Trizol). The lysates were then used immediately or stored at -30°C for subsequent RNA extraction. RNA was extracted using a high capacity cDNA reverse transcription kit, using instructions provided by the manufacturer. The cell homogenates were then transferred into Eppendorf tubes and were mixed with 0.2ml of chloroform per 1ml of Trizol reagent. The tubes were shaken vigorously by hand for 15 seconds, incubated for 2-3 minutes at room temperature, and centrifuged at 12,000*g* for 15 minutes at 4°C. The centrifuge used was the Centrifuge 5424R (Eppendorf). This caused the mixture to separate into three layers: a lower phenol-chloroform phase (red and clear), an interphase (colourless and cloudy) and the upper aqueous phase (clear and colourless). The aqueous phase, which contains all the RNA, was collected, whilst the interphase and organic layers were left undisturbed and discarded.

The RNA solution obtained was mixed with 0.5ml of 100% isopropanol per 1ml of Trizol reagent used in the homogenisation reaction. This mixture was incubated for 10 minutes at room temperature. In order to produce RNA pellets, this mixture was then centrifuged at 12,000*g* for 10 minutes at 4°C. Following this, the supernatant was removed from each Eppendorf tube, so that only the RNA pellet remained. The RNA pellet was subsequently washed with 1ml of 75% ethanol per 1ml of Trizol reagent used in the initial homogenisation. The tubes were then centrifuged for 5 minutes at a speed of 7,500*g*. Next, the wash was discarded, then the RNA pellet was allowed to air dry for at least 5 minutes, until free of visible liquid residue. 20µl of RNAse/DNAse free water was added to each sample, and the RNA resuspended into solution. Total RNA was measured using a POLARstar Omega microplate reader (BMG LABTECH GmbH, Offenberg, Germany). Raw data were uploaded from the POLARstar OMEGA software into a Microsoft Excel spreadsheet for analysis. The RNA and water volumes were adjusted depending on the RNA concentration, using the sample with the highest RNA concentration for the no reverse transcriptase control and normalising all other samples to this.

mRNA was then immediately converted to complementary DNA (cDNA) by reverse transcription. cDNA synthesis was undertaken using a reaction mixture (see **Table 3.2**) consisting of 2µl random primers, 2µl 10x buffer, 1µl dNTP mix (100mM), 1µl reverse transcriptase (50U/µl), 4µl water and 10µl RNA and water solution. The samples were kept on ice whilst the reagents were added. The total volume of the reaction mixture was 20µl. The reagent tubes were placed in the Techne TC-512 thermal cycler (Cole-Palmer, Staffordshire, UK) using the reverse transcription protocol (one hour at 37°C), to allow cDNA to be synthesised. If samples were used for PCR on the same day, they were stored at 4°C in the thermal cycler until required. If the PCR reaction was undertaken at another time, the sample tubes were returned to the freezer and stored at -30°C until required.

Figure 3.2 demonstrates the main steps in the processes of reverse transcription and real-time PCR.

Reagent	Volume (µl)
25X dNTP mix (100mM)	1
Reverse transcriptase (50U/µl)	1
Water	4
Random primers	2
10x buffer	2
Water and RNA solution	10
Total volume	20

Table 3.2 – The reagents used for cDNA synthesis, and their respective volumes.

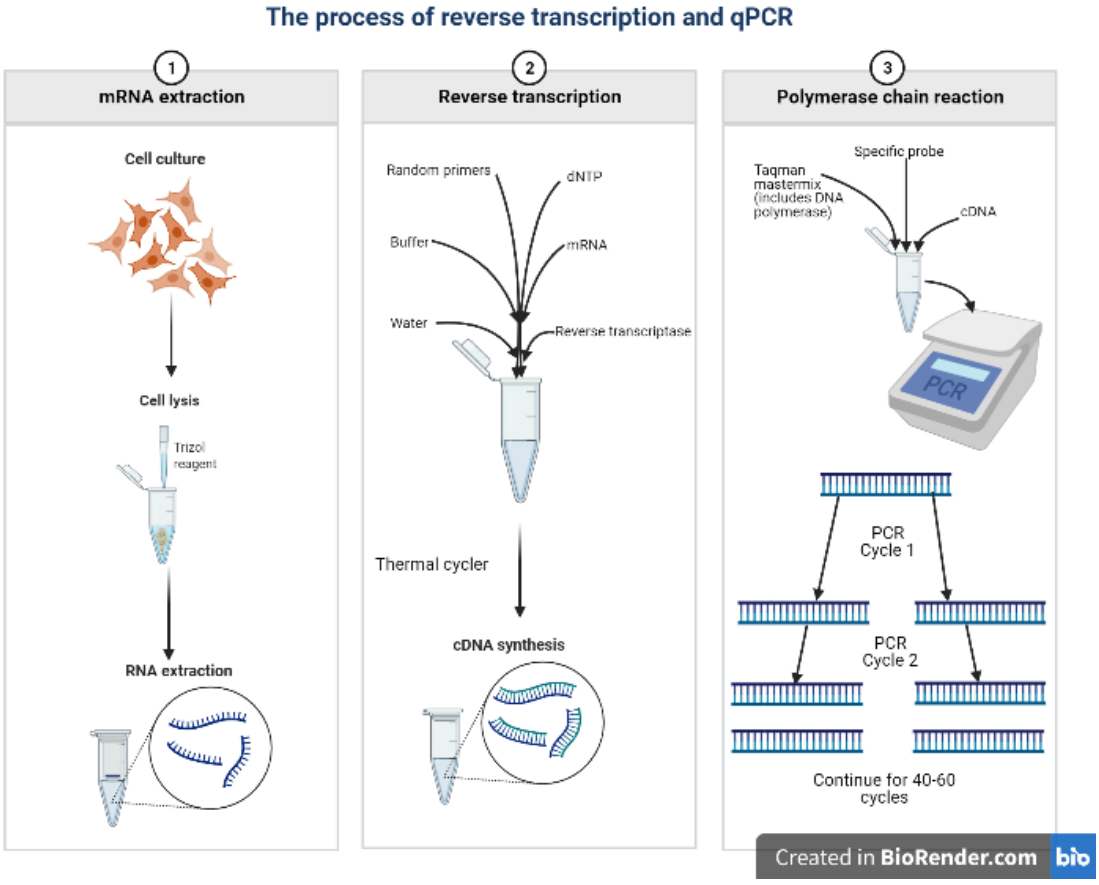


Figure 3.2 – A schematic showing the steps involved in mRNA extraction, reverse transcription and polymerase chain reaction.

First, cells (A549, BEAS2B or primary cells) were lysed using Trizol reagent, to release the mRNA. This mRNA was then quantified and mixed with several other reagents (see **Table 3.2**), to allow synthesis of complementary DNA. This cDNA was then amplified many times using real-time PCR. Figure adapted from "SARS-CoV-2 Genome Sequencing using Oxford Nanopore Technologies", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates[124].

3.2.7 Real time PCR

Quantitative qPCR was undertaken, using specific primers and probes for IL-6, ICAM-1 and CDHR-3, with L32 used as a control. Each run of PCR included a negative control sample, which contained all PCR reagents, except for cDNA, with an extra 2µl of water added instead. A 96 well plate PCR plate was used, with a duplicate of each sample being performed. Each well had 1µl of probe, 10µl of Mastermix and 7µl of water added, followed by 2µl of cDNA. The exception was the negative control, to which an extra 2µl of water was added, in place of cDNA. Four different PCR experiments were undertaken: measurement of the expression of CDHR3 following HRV-C infection (assay ID: Hs00541677_m1) and ICAM-1 expression following HRV-A infection (assay ID Hs00164932-m1), as well as IL-6 expression following HRV-A and HRV-C infection (assay ID Hs00174131_m1). Each experiment was undertaken in a different 96 well plate using the PrecisionPLUS qPCR Master Mix (PrimerDesign, Southampton, UK) in accordance with the manufacturer's instructions. The expression of each marker was compared to the expression of an internal control, L32 (assay ID: Hs00388301_m1). The Taqman assays used is shown in **Table 3.3**.

Primer/probe	Gene	Assay ID	Amplicon length
ICAM-1	ICAM1	Hs00164932-m1	87
CDHR3	CDHR3	Hs00541677_m1	124
IL-6	IL6	Hs00174131_m1	95
L32	MRPL32	Hs00388301_m1	81

Table 3.3 – Taqman assays used described according to their primer/probes, genes, assay ID and amplicon length

A 7300 Real-Time PCR System (Applied Biosystems, Forster City, California, USA) and corresponding software were used. A standard cycle protocol for PCR amplification (two minutes at 95°C followed by 45 cycles of 10 seconds at 95°C and one minute at 60°C) was followed. The probe used in each well was selected on the software and the amplification curve appraised for quality.

3.2.8 Statistical analysis

All experiments were undertaken at least three times. Comparison of data sets was undertaken using Microsoft Excel software. Results were presented as the mean data for each of the three samples in a table and the overall means ± standard deviations for each condition were plotted graphically. Graphs were produced using GraphPad Prism version 9.1.1 (GraphPad Software, San Diego, California, USA).

The expression of each gene was analysed using the comparative cycle threshold (Ct) method ($2^{-\Delta\Delta Ct}$), with housekeeping gene L32 used as the internal control, to which the Ct of the other transcripts was normalised. The steps taken are as follows:

1) Duplicates of Ct values for each transcript were averaged by adding together and dividing by the number of results. The mean Ct value for each of the three samples was then used to calculate an overall mean Ct value for each time point (n=3 in most cases, except where the result was undetermined).

2) Ct values were then standardised to the internal control gene (L32) by subtraction of the average control gene from the average target gene value, using the equation:

ΔCt = Ct (target mean) – Ct (L32 mean)

3) Next, the ΔCt values were corrected to the relative control, using this equation:

$\Delta\Delta Ct$ = mean ΔCt (infected group) – mean ΔCt (uninfected control)

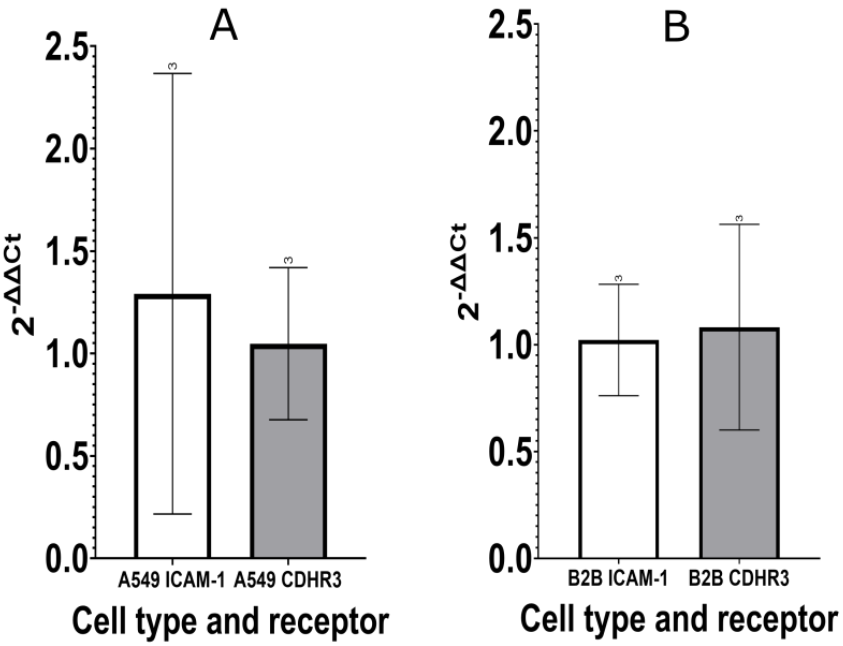
4) The fold change in target gene expression relative to the housekeeping gene, L32 is expressed as: relative gene expression = $2^{-\Delta\Delta Ct}$.

The mean and standard deviation values for expression for each gene were then used to graphically represent the results. The Kruskal-Wallis test was used to compare the $2^{-\Delta\Delta Ct}$ value for each time point to the control, as the results were non-parametric. A *P* value <0.05 was considered statistically significant.

3.3 Results

3.3.1 Baseline expression of ICAM-1 and CDHR3 in A549 and BEAS-2B cells

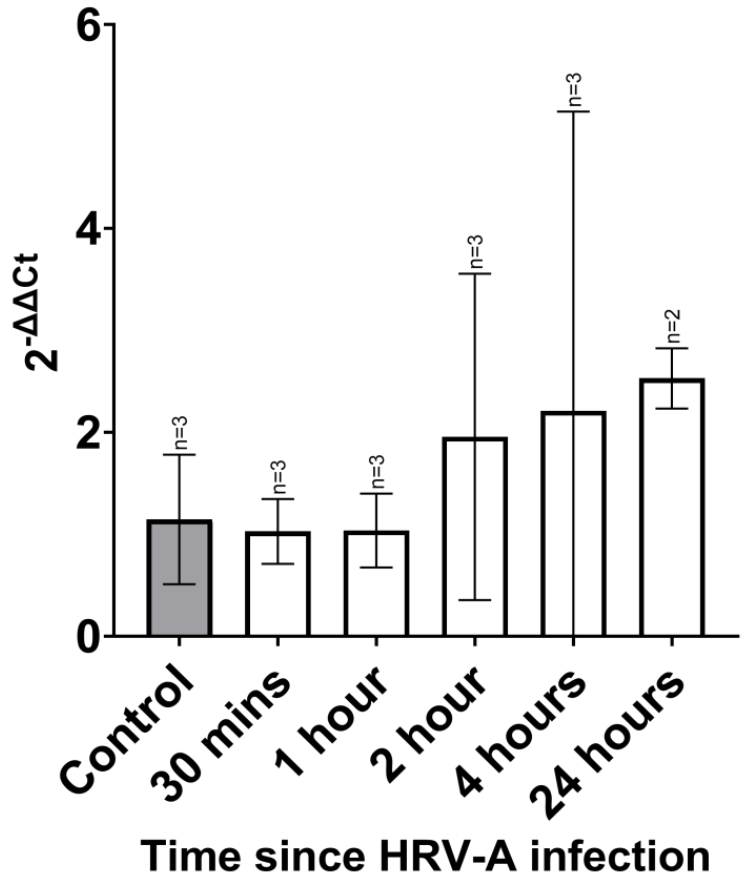
The baseline expression of the ICAM-1 and CDHR3 HRV receptors (in the absence of HRV infection) is similar and comparable between the BEAS-2B and A549 cell lines (**Figure 3.3)**.



A549 ICAM-1	A549 CDHR3	B2B ICAM-1	B2B CDHR3
2.5	1.1	0.8	1.2
1.0	0.7	1.3	1.5
0.4	1.4	1.0	0.6

Figure 3.3 – Constitutive expression of HRV receptor mRNA (ICAM-1 and CDHR3) by (A) A549 and (B) BEAS-2B cells. The table below shows the three $2^{-\Delta\Delta Ct}$ values for each condition.

3.3.2 Expression of ICAM-1 following HRV-A infection of BEAS-2B cells



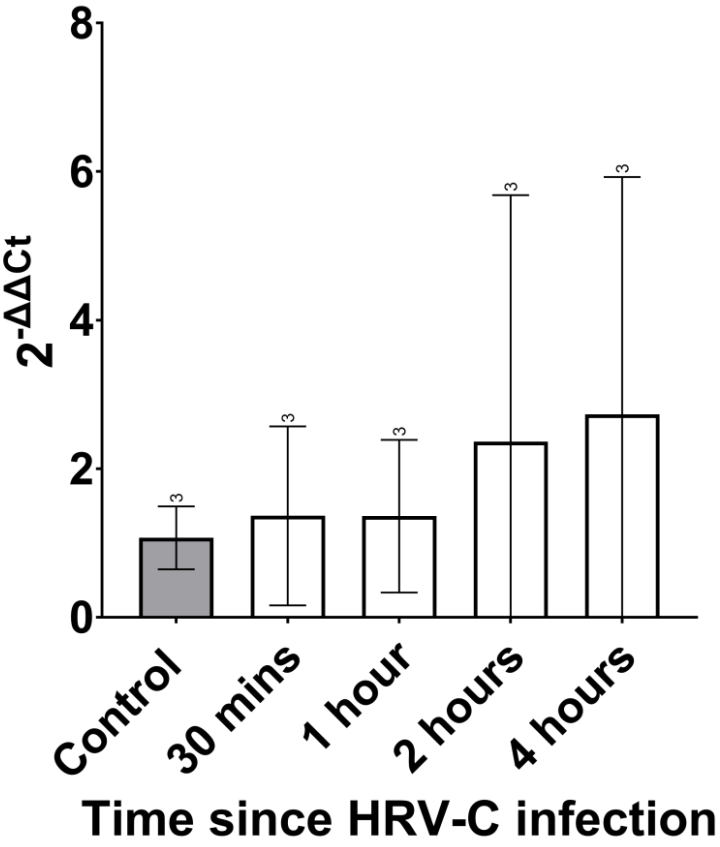
Control	30 mins	1 hour	2 hours	4 hours	24 hours
1.3	0.8	1.5	3.6	5.6	2.3
0.5	1.4	0.8	2.0	0.8	
1.7	0.9	0.9	0.4	0.2	2.7

Figure 3.4 – Expression of ICAM-1 mRNA in BEAS-2B cells infected with HRV-A: a 24 hour time course. The table below shows the $2^{-\Delta\Delta Ct}$ values for each condition.

ICAM-1 mRNA expression remained at a similar level to the control at each time point following HRV-A infection, until 2 hours, when it began to gradually increase until 24 hours (**Figure 3.4)**. One of the PCR reactions for the 24 hour samples was undetermined but all other time points have n=3 results. The mean (standard deviation or SD) expression at 2 hours (2.0[1.6]) was approximately double all earlier values, including the control (1.1[0.6]). The mean value increased to 2.2[2.9] at 4 hours and 2.5[0.3] at 24 hours. However, the standard deviations around these results were moderately large, so the differences

observed between each time point and the control were not statistically significant ($p>0.05$).

3.3.3 Expression of CDHR3 following HRV-C infection of BEAS-2B cells



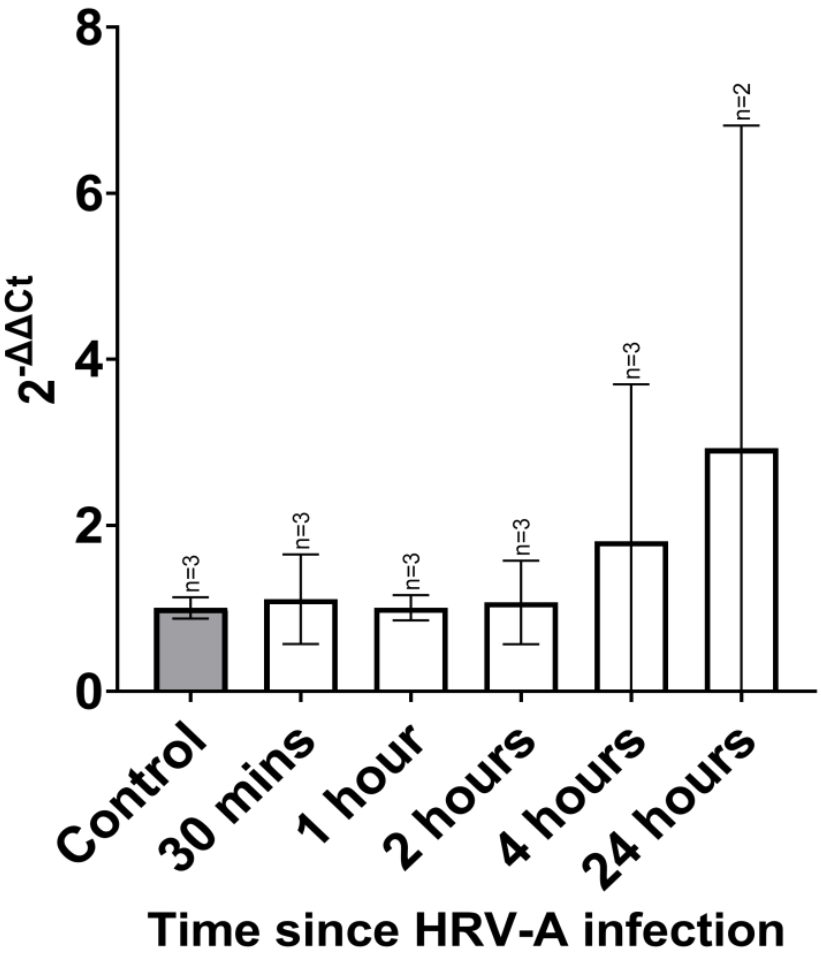
Control	30 mins	1 hour	2 hours	4 hours
0.6	1.0	1.4	6.2	6.3
1.3	0.4	0.3	0.7	0.1
1.3	2.7	2.3	0.2	1.8

Figure 3.5 – Expression of CDHR3 mRNA in BEAS-2B cells infected with HRV-C: a 4 hour time course. The table below shows the three $2^{-\Delta\Delta Ct}$ values for each condition.

CDHR3 mRNA expression remained similar to the control at each of the time points following HRV-C infection of BEAS-2B cells, with the exception of the 4 hour time point (**Figure 3.5)**. Unfortunately, all of the PCR reactions for the 24 hour samples and one of the 1 hour samples were undetermined. All other time points have n=3 results. The mean expression at 4 hours (2.7[3.2]) was approximately double all other values, including the control (1.1[0.4]). In spite of this, the standard deviations around these results were

relatively large, so the differences observed between this time point and the control were not statistically significant (p>0.05).

3.3.4 Expression of IL-6 following HRV-A infection of BEAS-2B cells



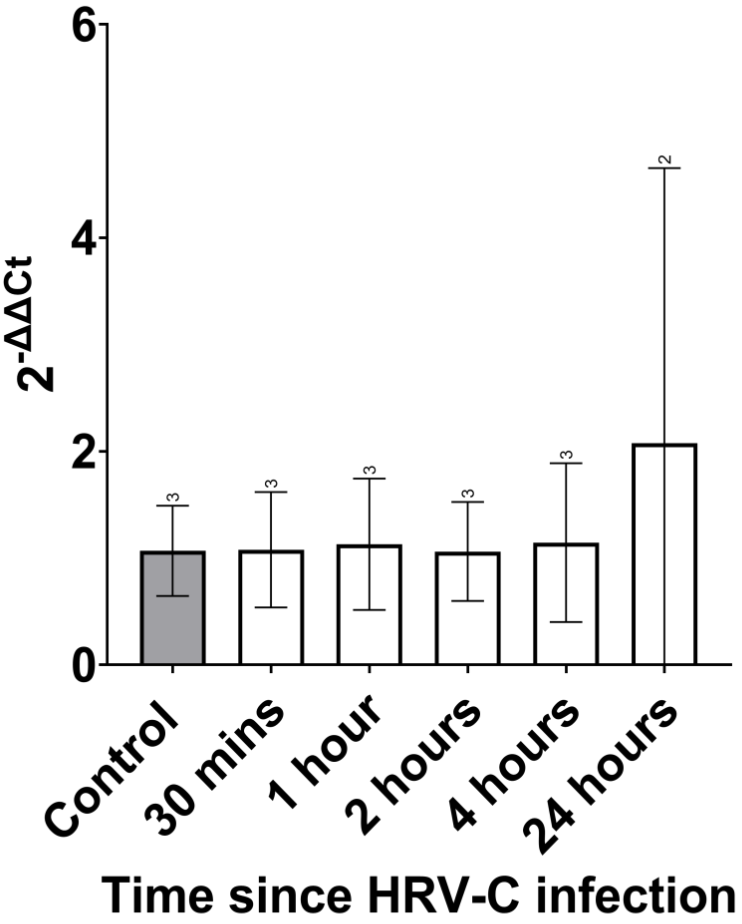
Control	30 mins	1 hour	2 hours	4 hours	24 hours
1.1	0.5	1.1	1.6	1.3	5.7
0.9	1.5	1.1	0.9	3.9	
1.1	1.3	0.8	0.7	0.2	0.2

Figure 3.6 – Expression of IL-6 mRNA in BEAS-2B cells infected with HRV-A: a 24 hour time course. The table below shows the $2^{-\Delta\Delta Ct}$ values available for each time point.

Mean expression of IL-6 mRNA remained at a similar level to the control at each of the early time points following HRV-C infection, then increasing at the 4 hour time point, to a peak at 24 hours (**Figure 3.6)**. One of the 24 hour samples was undetermined, however all

other time points have n=3 results. The mean expression at 4 hours (1.8[1.9]) was nearly double all of the other values, including the control (1.0[0.1]). The expression increased further to a peak at 24 hours (2.9[3.9]). The standard deviations around these results were relatively large, so the differences observed between each time point and the control were not statistically significant (p>0.05).

3.3.5 Expression of IL-6 following HRV-C infection of BEAS-2B cells



Control	30 mins	1 hour	2 hours	4 hours	24 hours
0.6	0.8	1.2	1.6	2.0	
1.3	1.7	0.5	0.9	0.6	3.9
1.3	0.8	1.7	0.7	0.9	0.3

Figure 3.7 – Expression of IL-6 mRNA in BEAS-2B cells infected with HRV-C: a 24 hour time course. The table below shows the $2^{-\Delta\Delta Ct}$ values available for each condition.

IL-6 mRNA expression remained at a similar level to the control at each of the time points following HRV-C infection, with the exception of the 24 hour time point (**Figure 3.7**). The

mean expression at 24 hours (2.1[2.6]) was almost double all of the other values, including the control (1.1[0.5]). There were large standard deviations around these results, so the difference observed between the 24 hour time point and the control is not statistically significant ($p>0.05$).

3.3.6 Relationship between IL-6 and rhinovirus receptor expression in epithelial cells infected with HRV-A and HRV-C

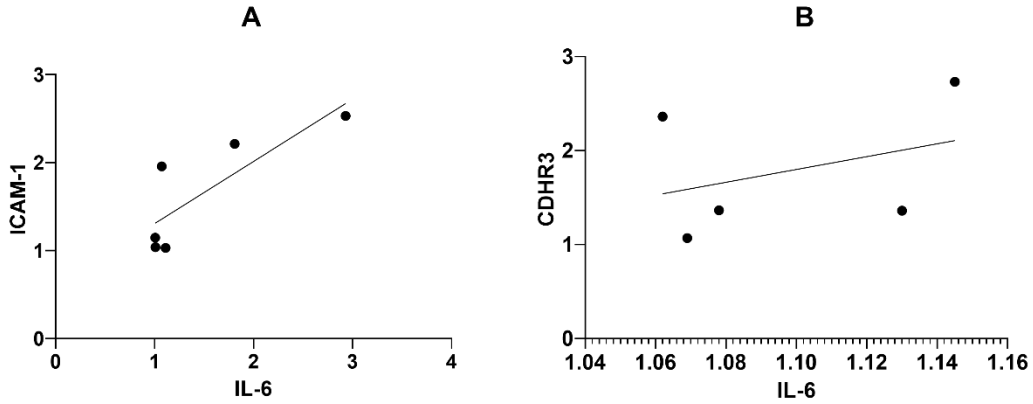


Figure 3.8 – Correlation between the expression of IL-6 and rhinovirus receptors in BEAS-2B cells A) Relationship between IL-6 and ICAM-1 mRNA expression following HRV-A infection B) Relationship between IL-6 and CDHR3 mRNA expression following HRV-C infection

There was a moderate positive correlation between the expression of IL-6 and ICAM-1 following HRV-A infection of BEAS-2B cells (r^2=0.67), **see Figure 3.8**. On the other hand, there was only a weakly positive correlation between IL-6 and CDHR3 expression by BEAS-2B cells following HRV-C infection (r^2 =0.13). However, due to the 24 hour time point for CDHR3 expression being undetermined, this relationship cannot be fully appraised.

Chapter 4: Retrospective medical record review exploring the relationship between demographics, clinical outcomes and social characteristics for viral-induced wheeze and asthma

4.1 Background

The 2020 population projection for Liverpool Local Authority Area is 502326[289]. Liverpool is currently working to become a UNICEF Child Friendly City, after being accepted in May 2019[290], an initiative which involves working to improve the health, wellbeing and life chances of children across the city[290]. Alder Hey Children's Hospital is a specialist paediatric teaching hospital based in Liverpool, with 270 beds, including 48 critical care beds[291]. Every year, the hospital cares for over 330,000 children, making it one of the largest children's hospitals in Europe[292]. In addition, the AHCH ED is one of the busiest children's EDs in the UK, with approximately 60,000 attendances each year[293].

The Index of Multiple Deprivation (IMD) is the official measure of relative deprivation in England[294]. The most recent version is IMD2019, which measures seven domains, including: education, health, income, crime and living environment[294]. IMD can be used to compare deprivation levels in small areas across England, as well as to identify changes over time[294]. Furthermore, the IMD is better for use in non-working age populations than some other measures, as it is not solely reliant on income or employment[295].

Whilst there have been several studies exploring the link between prevalence of preschool wheeze or asthma and socioeconomic status,[114, 115, 296] there is little data relating the Index of Multiple Deprivation to paediatric acute wheezing episodes. In particular, data relating to the number of attendances and severity of disease in the preschool age group is deficient, with some previous studies only including individuals aged over 5 years[295, 297]. There has also been limited comparison of the preschool and school aged groups. Thus, it

would be useful to investigate if there are any inequalities in acute admissions and clinical outcomes, across the paediatric age group, including their relationship to the IMD2019.

NO_2 is an important air pollutant, with multitudinous reported health effects. These include increasing the risk of cardiovascular and respiratory disease[298]. Positive associations between 24 hour NO_2 levels and hospital admissions for respiratory disease, including asthma and COPD, have also been noted[298]. However, when appreciating this relationship, it is important to remember that emissions of NO_2 are often closely linked to those of other traffic-related pollutants, such as particulate matter[298, 299]. As such, it can be difficult to definitively quantify the effect size of individual pollutants[298].

The current maximum level of NO_2 recommended by WHO, and the Department for Environment, Food and Rural Affairs (DEFRA), is an annual mean of 40μg/m^3, and an Air Quality Management Area (AQMA) needs to be declared if this is not achieved[117, 300]. Despite this target, the British Lung Foundation reports that 90% of local authorities are exceeding this target in at least one location, according to maximum annual mean roadside levels[301]. This includes Liverpool which is currently designated an AQMA[302]. In addition, there are significant adverse health sequalae associated with NO_2 concentrations at the current recommended level, leading some scientists to propose a lower target and better enforcement of current guidelines[301]. A sophisticated study by Williams *et al*., published in the Lancet, modelled four different scenarios up until 2050, varying depending on environmental policy. This study showed the sizable impact of reductions in NO_2 emissions[299]. If low greenhouse gas policies are employed between 2011 and 2050, over 7 million life-years could potentially be saved[299].

Liverpool has an Air Quality Action Plan (AQAP), which was published in 2007 and updated in 2011[137, 302]. The AQAP highlighted several options to improve air quality, especially

emissions of oxides of nitrogen (NO_x), including encouraging alternative transport methods and better development planning[302].

Air pollution and its links to respiratory health has been recently reported by the media because of the death of a 9 year old girl, in February 2013[303]. Ella Adoo-Kissi-Debrah had severe asthma and lived close to a highly congested road in Lewisham, London[303]. It was ruled by a coroner in December 2020 that air pollution exposure was a cause of death, which is thought to be the first case of this sort[303]. Following this ruling, the Chief Executive of Asthma UK stated "this verdict sets the precedent for a seismic shift in the pace and extent to which the government, local authorities, and clinicians must now work together to tackle the country's air pollution health crisis"[303].

Further to this ruling, on the 20th April 2021, the coroner involved in the case sent a 'report to prevent future deaths' to 14 different government departments and medical organisations, including NICE and the Royal College of Paediatrics and Child Health (RCPCH)[304]. This report listed several concerns and suggested actions[304]. These suggestions included: making the WHO air quality guidelines legally binding targets, improving public awareness of air pollution, and increasing the availability of air quality data[304].

Past studies of the association between preschool wheeze/asthma and air pollution have largely neglected preschool children and few have reported on the associated clinical outcomes. Therefore, this study will provide important evidence regarding the association between NO_2 air pollution and acute wheezing episodes among children aged 2-16 years, in the Liverpool area, and explore the corresponding clinical outcomes.

4.1.1 Aims

The work presented in this chapter on Alder Hey Children's Hospital attendance with viral induced wheeze and asthma aims:

- To describe patient demographics and investigate their association with clinical outcomes.

- To compare subgroups of the cohort according to age, sex and other demographic factors and compare the associated clinical outcome measures.

- To highlight temporal trends in hospital admissions

- To explore the relationships between social characteristics (air quality and deprivation), number of admissions and corresponding patient outcomes.

4.1.2 Hypotheses

Hypothesis 1: Children living in areas with higher levels of deprivation are more likely to be admitted and have worse clinical outcomes

Hypothesis 2: Children living in areas with higher levels of air pollution are more likely to be admitted and have worse clinical outcomes than children in less polluted areas

4.2 Methods

4.2.1 Study design and location

A retrospective observational study of children living within a Liverpool postcode aged 2-16 years admitted to Alder Hey Children's Hospital with VIW or asthma, between 1st September 2015 and 31st August 2020, was undertaken.

4.2.2 Case Definition

Details of admissions to AHCH, due to viral-induced wheeze and acute asthma, in children with a Liverpool postcode aged 2-16 years was obtained from the hospital audit and IT departments. Patients with a non-Liverpool postcode at the time of admission were excluded. This age range was chosen as children aged less than 2 years are more likely to have bronchiolitis[44]. These events were defined as all hospitalizations with a diagnosis of acute exacerbation of asthma or viral-induced wheeze in the discharge report, between September 2015 and August 2020. The codes used from OPCS 4th Edition were B349 (Viral infection, unspecified), J46X (Status asthmaticus), J459 (Asthma, unspecified), J450 (Predominantly allergic asthma) and R062 (wheezing). Patients with wheezing illness due to

bronchiolitis (codes J210-J219) were not eligible for inclusion in this analysis. Moreover, patients who had outpatient treatment, but were not admitted to the hospital, were excluded. Any patients who died within the data collection period were excluded from the analysis, as these patients were likely to have complex medical needs and readmission data for the full data collection period is not available.

4.2.3 Data collection

Patient data were collected from electronic patient records. The data recorded within a data extraction form included: date and time of admission, date of birth, date of death, sex, postcode, date and time of discharge, diagnosis, number of patient episodes in spell, requirement of oxygen (determined by Paediatric Early Warning Score), critical care admission requirement and respiratory readmissions within 12 months of initial admission. The age of each patient upon admission was calculated by finding the difference between each individual's date of birth and their date of admission. Length of stay was collected in full and partial days using the dates and times of admission and discharge from the hospital. For most of the analyses, all attendances were included, regardless of their length of stay. Further to this, an additional analysis which distinguished between admissions lasting less than six hours and those lasting 6 or more hours was undertaken. This was because admissions lasting less than six hours were generally to the medical observation ward 'attached' to the emergency department. In all other analyses, whenever admissions are mentioned, this term refers to all patients, regardless of length of stay.

Critical care admissions included patients who were admitted to the high dependency unit (HDU) or paediatric intensive care unit during their hospital day (directly from the ED or transferred from inpatient ward). All respiratory readmissions (OPCS codes J00-J99) within 12 months of the index admission were identified. The data for each patient across multiple admissions to AHCH was linked using unique patient identification numbers.

Patients were grouped according to their age at first admission into two age bands: 2-6 years or 7-16 years. This is because preschool wheezing illness is often considered a distinct entity to school-aged asthma[3]. Furthermore, children within these age bands are likely to have different lifestyles and exposures, with older children more likely to attend school and take responsibility for any medication they require.

The English Index of Multiple Deprivation 2019 was used to assign each patient a deprivation rank and decile, according to their postcode[294]. There is a postcode mapper function which can be used to identify the lower-layer super output area where an individual resides[294]. This data can then be used to compare the outcomes of people who live in different areas of deprivation.

Air pollution data for the same period as the admissions data was obtained in collaboration with Liverpool City Council. NO_2 was the only pollutant that was included in this analysis due to time constraints. It was chosen over other pollutants, such as particulate matter, due to the availability of a large network of monitoring stations. Moreover, it has previously been suggested that high NO_2 exposure in the week before a childhood respiratory infection is linked to more severe viral-induced asthma exacerbations[305]. During the period 2015-2020, there were two air quality monitoring stations in Liverpool, under the management of DEFRA[306]. The details of these sites are as follows:

- Speke – this urban background site, 7.7 miles South East of the city centre, is one of 171 Automatic Rural and Urban Network (AURN) sites across the UK[307]. This AURN measures many pollutants, including NO_x[307] and it opened in 2003[300].
- Queen's Drive – this urban kerbside site is approximately 4.3 miles to the North of the city centre measured NO_x as part of the AURN since its installation in January 2008[306]. This site was closed in November 2016, so only data for the period September 2015 to November 2016 was available for this analysis.

Environmental factors, such as modelled wind speed, wind direction and temperature, are also measured at these sites[307]. This data is accessible online, via the Air Quality in England website[308]. Data was also sourced from the non-automatic diffusion tubes in the Liverpool city area. Passive diffusion tubes measure ambient nitrogen dioxide and they are useful for identifying which areas have a high NO_2 concentration[309]. In total, there are 73 diffusion tubes in the Liverpool City Council AQMA[306], arranged in five groups: Central, North, South and the number 10 and 14 bus routes[306]. There are also 10 PDTs at Liverpool John Lennon Airport[306]. PDT sites were connected using lines to form polygons, and the patients with postcodes within each polygon were recorded as having the same NO_2 exposure.

4.2.4 Statistical analyses

Excel (Microsoft Corp., Washington, USA) was used to sort data and obtain some descriptive statistics. GraphPad Prism 9.1.1 software was used to undertake statistical analysis and generate the graphs.

In order to allow comprehensive data analysis, the completeness of data for each of the variables of interest was evaluated. The one sample Kolmogorov-Smirnov test was used to test for a normal distribution. A significant Kolmogorov-Smirnov test result shows that a variable is not normally distributed, whereas a non-significant Kolmogorov-Smirnov test result suggests a normal distribution. Most of the variables measured were not normally distributed, so predominantly non-parametric tests were selected.

Descriptive statistics chosen were the mean values and standard deviations for parametric continuous variables and median and interquartile range values for non-parametric values. Comparisons between two groups were made using the Chi-squared test for nominal data, the independent samples t-test for parametric scale data and the Mann-Whitney U-test for non-parametric scale data. The Kruskal-Wallis test was used to compare more than two groups when normality could not be assumed. Simple regression analysis was used to

assess the relationship between two continuous variables and Spearman's correlation was used to measure the correlation. Binary logistic regression was used to quantify the relationship between scale and nominal variables. A p value of <0.05 was deemed statistically significant and all statistical tests were two tailed.

4.3 Results

4.3.1 Overall patient demographics

The dataset included 2493 individual children aged between 2 and 16 years. This dataset comprised 907 female and 1586 male patients. The age split of the individuals admitted (at time of their first admission) was 1945 in the 2-6 years age band and 548 in the 7-16 years age band.

There was a total of 4263 admissions in these individuals during the data collection period, which were used for all other analyses. The greatest proportion of admissions were in children aged 2-3 years (28.2%), followed by 3-4 years (19.3%) and 4-5 years (12.6%), and then gradually reducing to a lower proportion for each age above 6 years (**Figure 4.1)**. There were significantly more admissions of males (2739) than females (1524) for viral-induced wheeze or asthma (see **Figure 4.1**).

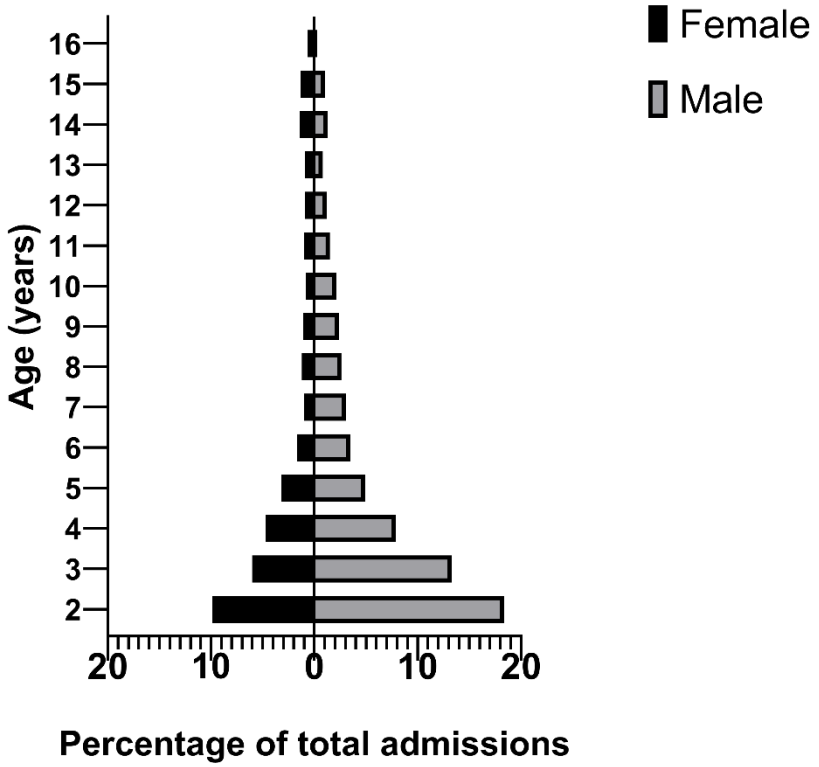


Figure 4.1 – Population pyramid showing the percentage of total admissions comprised by each sex and age group. For instance, age 2 years includes all children aged between 2 years and 2 years 364 days.

The median age at first admission was 3.74 years (interquartile range (IQR) = 3.81 years). Meanwhile, the median age for all admissions (including readmissions) was 4.16 years (IQR = 4.45 years). In total, there were 3134 admissions in the 2-6 year age band and 1129 admissions in the 7-16 year age band (see **Figure 4.2**). The median age for all female admissions was 4.39 years (IQR = 5.39), whereas the median age for all male admissions was 4.05 years (IQR = 4.19). There was a statistically significant difference in the age at admission for the male and female patients (p=0.0006). The male to female ratio was significantly different for the two age bands; 65.3% of admissions of patients aged 2-6 years were male and 61.4% of admissions aged 7-16 years were male, p=0.019.

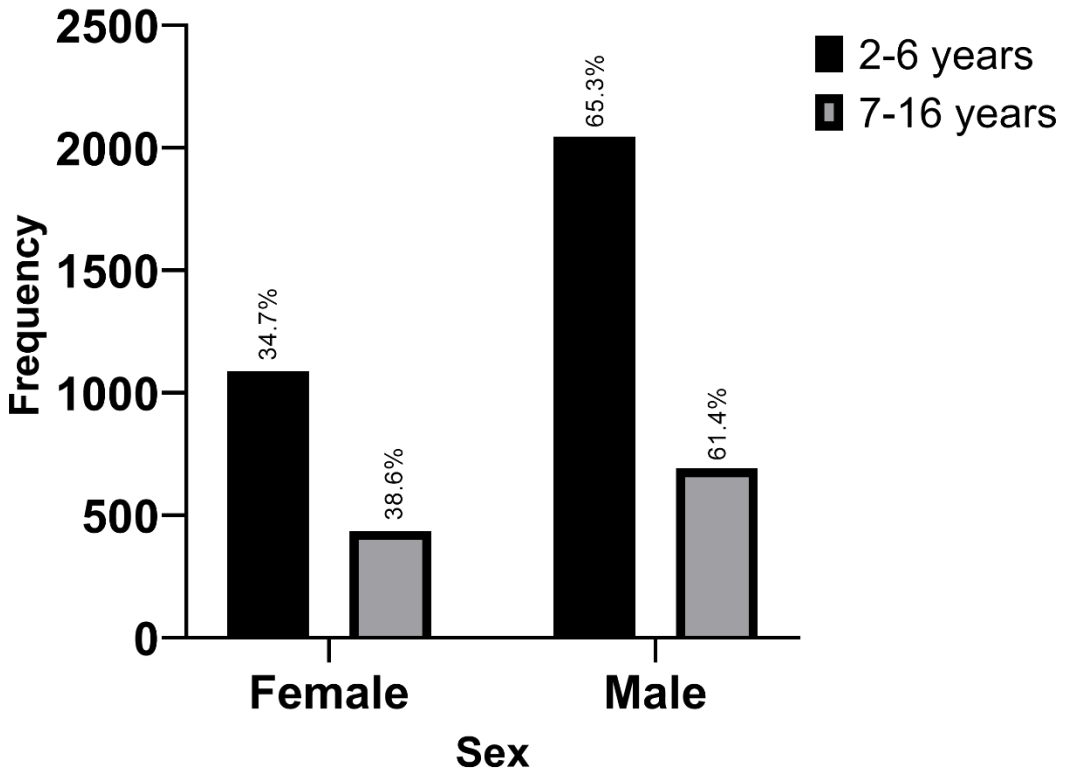


Figure 4.2 - The age band and sex of each patient admitted to AHCH for VIW or asthma exacerbations (percentage of each age band is shown above the bars). This data may include multiple admissions of some patients.

Simple linear regression was carried out to explore the relationship between age at admission (years) and length of stay (days). There was a weakly positive linear relationship between the two, which was confirmed with a Spearman's correlation coefficient of 0.1416. The linear regression showed a significant association between age and length of stay ($p<0.0001$). In addition, the slope coefficient for age was 0.05689, so the length of stay increases by 0.05689 days for each year of age. The R^2 value was 0.02006 so 2.006% of the variability in length of stay can be attributed to age at admission.

4.3.2 Temporal trends in admissions

318 admissions were in 2015 (between 1st September and 31st December), 1125 were in 2016, 882 were in 2017, 941 were in 2018, 798 were in 2019 and 199 were in 2020 (between 1st January and 31st August). The most common month of admission was September (611 admissions, 14.3%), followed by November (531, 12.4%) and October (518, 12.2%), see **Figure 4.3** and **Figure 4.4**. Following this autumn peak, the number of admissions were fluctuated at a lower level, before reducing further during the summer, with the lowest number of admissions occurring in August (151, 3.54%).

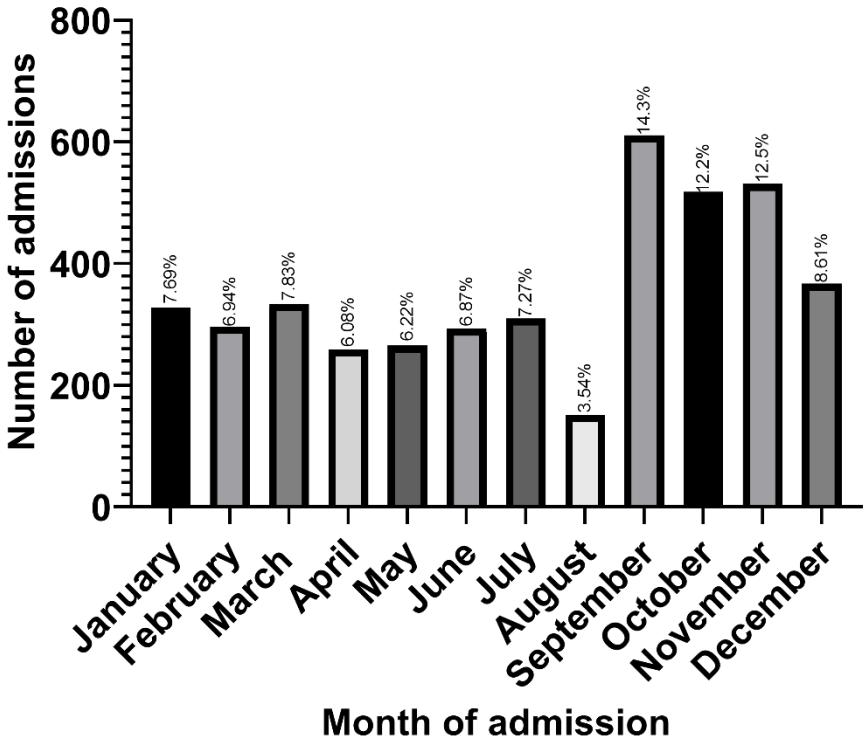


Figure 4.3 Total monthly admissions for VIW and asthma at AHCH between 1st September 2015 and 31st August 2020).

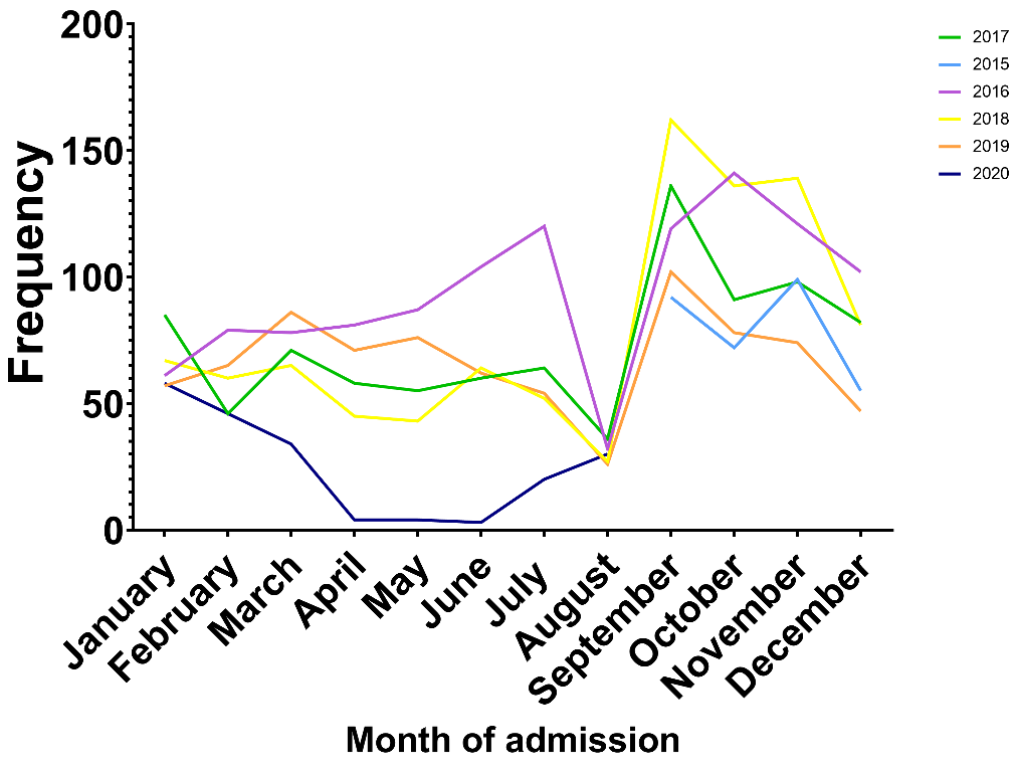


Figure 4.4 – Number of admissions per month for each year

The median total number of admissions on each day of the week was 591 people (IQR = 106.5). The day with the highest number of admissions was Monday (n=721, 16.9%). This was closely followed by Tuesday (n=683, 16.0%). The next most frequent day of admission was Sunday (n=637, 14.9%). The other days of the week, Wednesday to Saturday, showed a substantially lower number of total admissions. Thursday was the least frequent day of admission (n=524, 12.3%), demonstrated by **Figure 4.5**. The daily variation in the number of admissions per hour to AHCH was significant, p=0.0002.

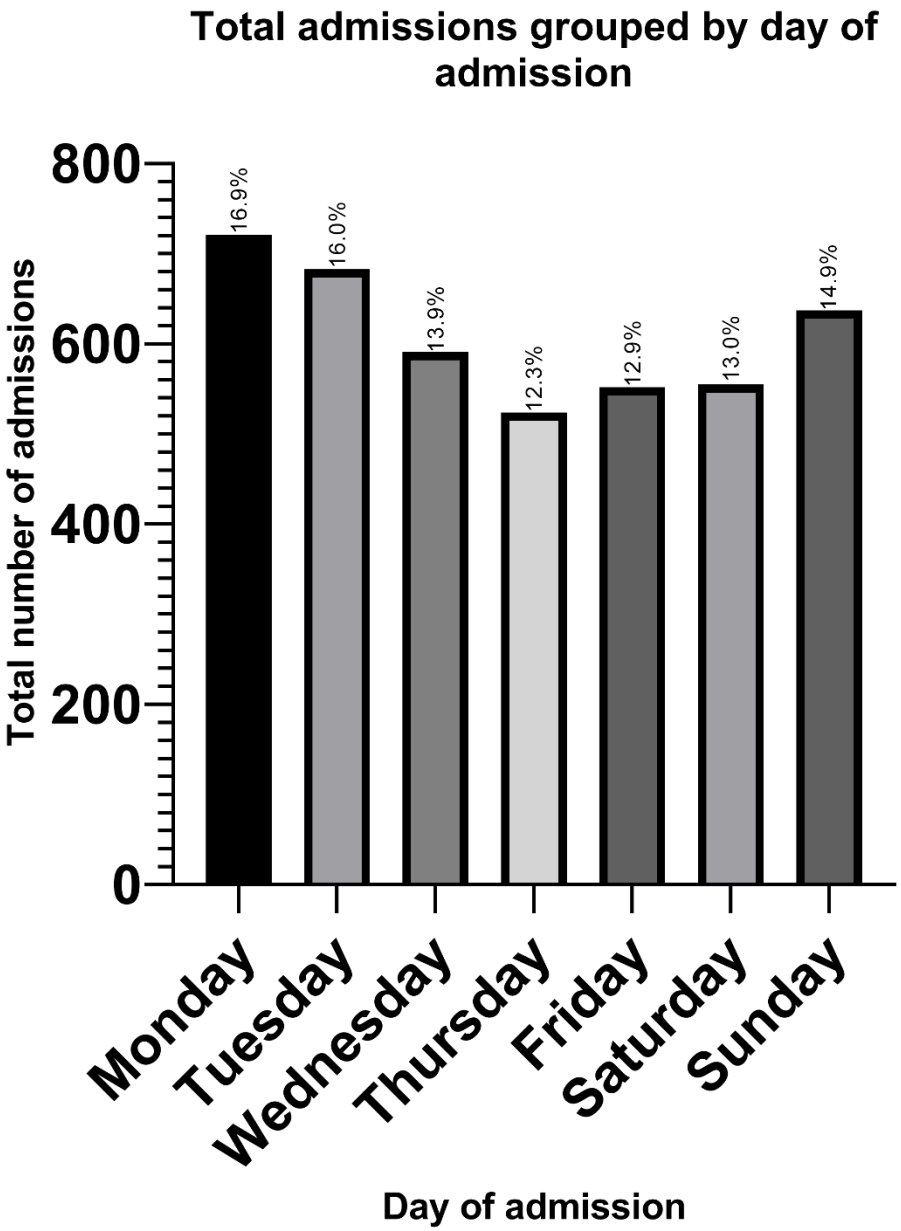


Figure 4.5 – Total admissions for VIW/asthma according to the day of admission.

Further analysis of the admission times revealed a diurnal variation in the number of admissions (see **Figure 4.6)**. The number of admissions was relatively similar for most hours of the day, but decreased significantly after midnight until approximately 7am, where they reached their lowest level, with only 65 admissions recorded between 7 and 8am (1.52%). Following this, the number of admissions rose progressively throughout the day, reaching a peak at between 3 and 4pm (n=243, 5.70%). This was followed by a slight trough, before

the highest peak between 9pm and 10pm (n=253, 5.93%) which was followed by another gradual decline, before a final peak between midnight and 1am (n=241, 5.65%).

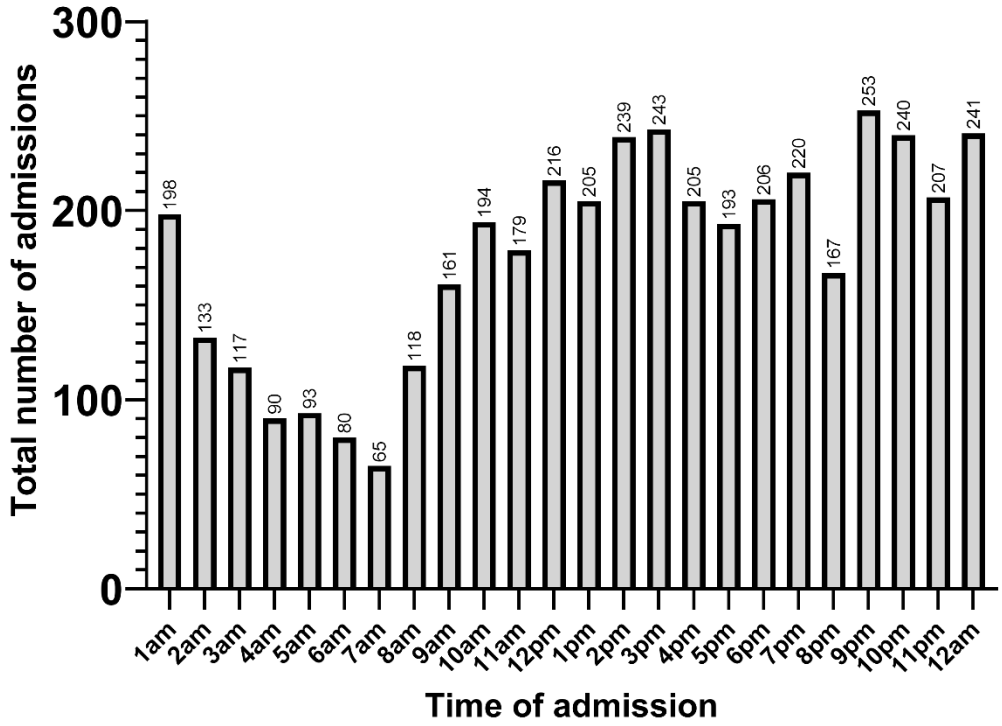


Figure 4.6 – Diurnal variation in admission frequency at AHCH for VIW and asthma. Each bar represents an hour, for example 1am represents 01:00 to 01:59am.

4.3.3 Clinical Outcome Data

The median length of stay for all admitted children was 0.312 days (IQR = 1.35 days). In total, 2811 admissions (65.9%) had a length of stay of less than 24 hours, 805 (18.9%) had a length of stay between 24 and 48 hours, 558 (13.1%) had a length of stay between 2 and 5 days and 89 (2.09%) had a length of stay more than 5 days (see **Figure 4.7**). The length of stay was significantly longer for female patients than male patients (p=0.0005). The length of stay was not significantly different for the two age bands studied (2-6 years and 7-16 years, p=0.2495).

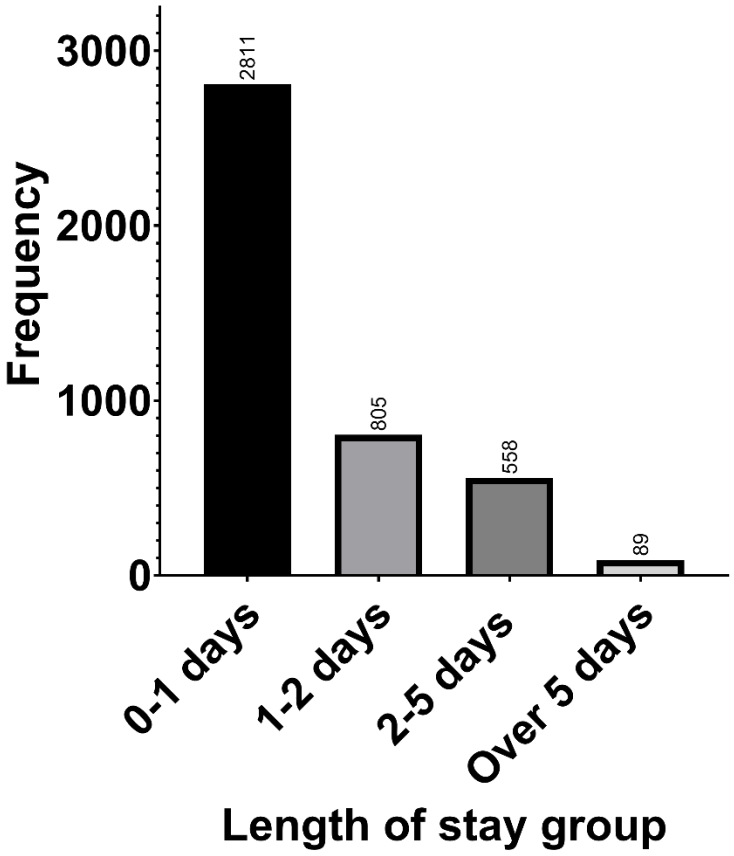


Figure 4.7 – Length of stay group for each of the admissions included in the data analysis.

The majority of patients in both the 2-6 years and 7-16 years age bands did not require oxygen or critical care admission during their hospital stay (**Figure 4.8).** In fact, 1271 (29.8%) of the patients admitted required oxygen in this time period (29.8%). Female patients were significantly more likely to require oxygen than male patients (p=0.0324). There was no statistically significant difference in oxygen requirement between age bands (p=0.2677).

In total, 63 individual patients required 72 critical care admissions (1.69% of admissions) at some point during at least one patient stay. There were 42 males and 30 females admitted to critical care. The median age of these children was 9.04 years on the admission date (IQR=7.7564). Thirty admissions were in the 2-6 years age band, and 42 were in the 7-16

years age band. The median length of stay for this group was 4.09 days (IQR=6.4311). This is significantly different from those who did not require critical care admission (p<0.0001). Forty-three (59.7%) of these children were readmitted to AHCH with a respiratory diagnosis within 12 months of the initial admission date. Critical care admission was not significantly associated with sex (p=0.2907). There was, however, a statistically significant difference between critical care admission and age band, with 7-16 years associated with higher odds of critical care admission (p<0.0001). The median critical care length of stay was 26.5 hours (IQR = 23.4).

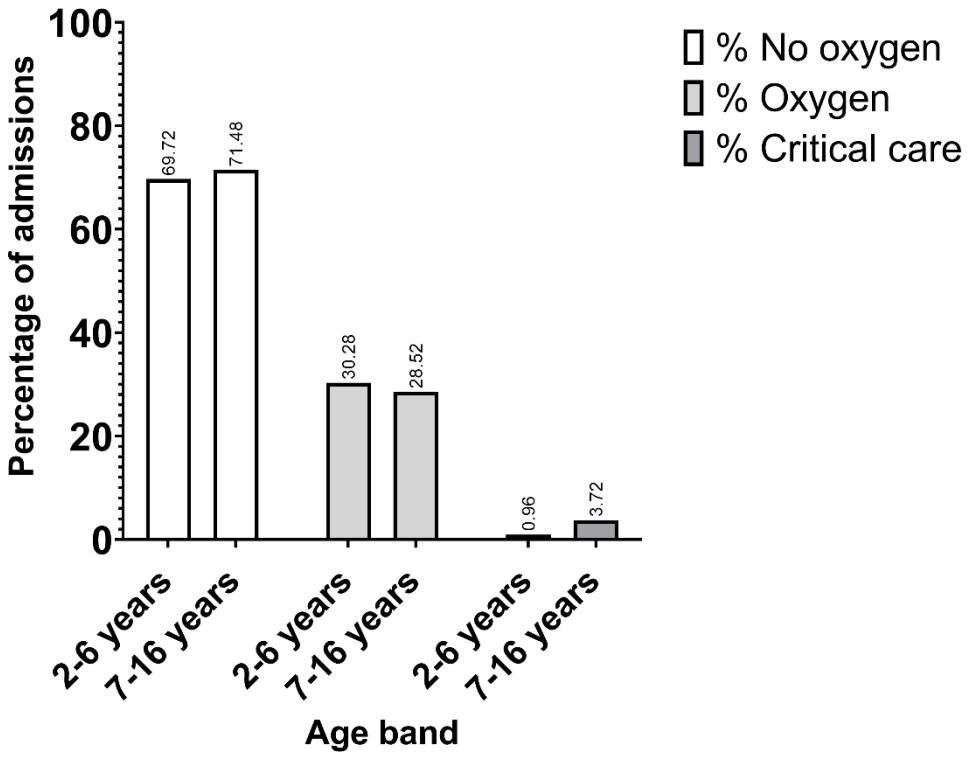


Figure 4.8 – Percentage of each age band that required each oxygen or critical care support during their admission.

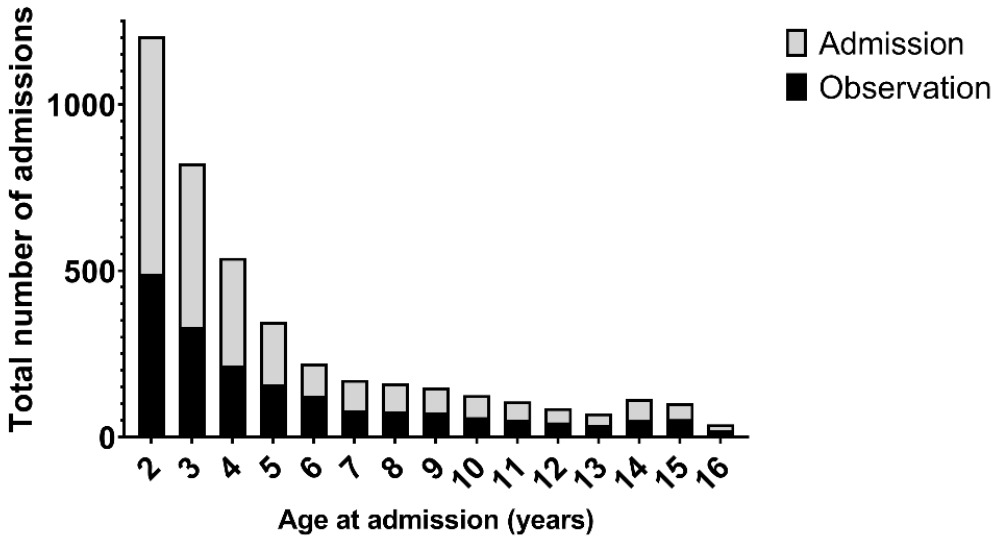


Figure 4.9 – Number of patients observed and admitted during the period of data collection, by age on admission date.

The rate of respiratory readmissions within 12 months of initial admission was very high, at 2960 (69.4%). Children who were admitted to the critical care unit (CCU) during their stay appeared less likely to be readmitted in the 12 months following their admission (with a respiratory diagnosis) than those who did not (59.7% vs 69.6%), although this was not statistically significant (p=0.3333). In addition, children who received oxygen at some point during their admission appeared less likely to be readmitted within 12 months (49.9% vs 77.7%), although this was not significant (p=0.3333). The risk of readmission was significantly associated with sex of the child. Although the difference was small, male patients were significantly more likely to be readmitted within the subsequent 12 months (70.6% vs 67.4%, p=0.0305). There was no significant relationship between age band and risk of readmission with a respiratory diagnosis within 12 months (p=0.6467).

4.3.4 Sex differences in admissions and patient outcomes

There were 2046 admissions of male patients in the 2-6 year age band (74.7% of all male admissions) and 693 male admissions in the 7-16 age band (25.3%). In contrast, there were 1088 admissions of female patients aged 2-6 years (71.4% of females) and 436 female admissions aged 7-16 years (28.6%). A comparison of male and female patients is

presented in **Table 4.1**. The significant differences between male and female patients are the age at admission, length of stay, proportion requiring oxygen and proportion requiring critical care. There was no significant difference between the IMD decile or rank, or percentage requiring critical care admission between male and female patients. **Figure 4.10** compares the frequency of each length of stay group by sex.

	Male (n=2739)	Female (n=1524)	P value	Significance
Age, median (IQR)	4.05 (4.19)	4.39 (5.39)	0.0006	S
IMD decile, median (IQR)	1.00 (2.00)	1.00 (2.00)	0.2108	NS
IMD rank, median (IQR)	1822 (7116)	1788 (6214)	0.8890	NS
Length of stay (days), median (IQR)	0.299 (1.23)	0.357 (1.51)	0.0005	S
% length of stay less than 24 hours (n)	68.1 (1864)	62.1 (947)		
% length of stay 24-48 hours (n)	18.7 (511)	19.3 (294)		
% length of stay of 2-5 days (n)	11.7 (320)	15.6 (238)		
% length of stay of 5 days or over (n)	1.61 (44)	2.95 (45)		
% requiring critical care admission (n)	1.53 (42)	1.97 (30)	0.2907	NS
% requiring oxygen (n)	28.7 (786)	31.8 (485)	0.0324	S
% with at least one respiratory readmission within 12 months (n)	70.6 (1933)	67.4 (1027)	0.0305	S

Table 4.1 – Demographic Features and Clinical Outcome Data of Children aged 2-16 years admitted with acute VIW or asthma, by sex. S = significant at p<0.05, NS = not significant.

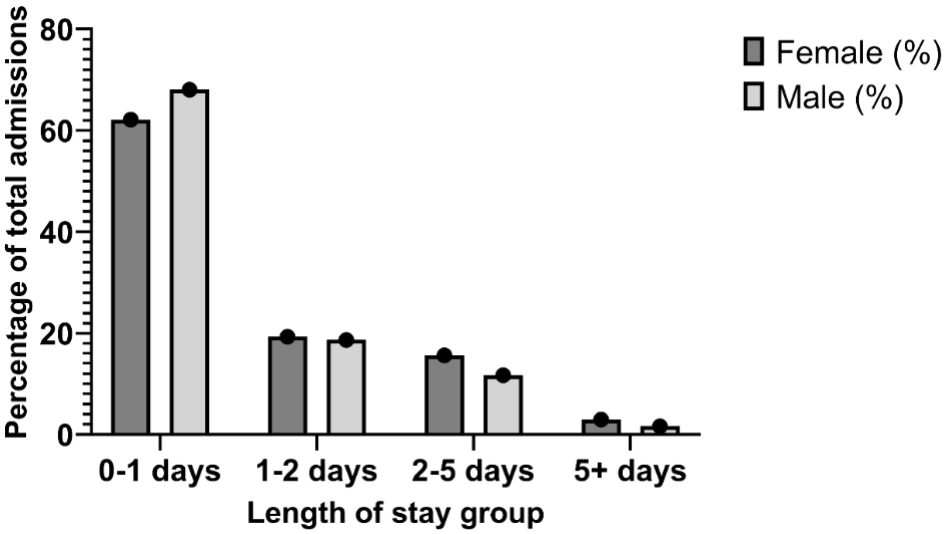


Figure 4.10 – Length of stay group for each admission, as a percentage of total admissions for each sex.

4.3.5 Age differences in admissions and patient outcomes

A comparison of the age bands is presented in **Table 4.2**. The only statistically significant difference between the age bands is the percentage requiring critical care admission, which was more likely in the 7-16 years age band. **Figure 4.11** shows the length of stay group for each admission, as a percentage of the total admissions for each age band.

	2-6 years (n=3134)	7-16 years (n=1129)	P value	Significance
IMD decile, median (IQR)	1822 (6726)	1822 (6329)	0.7014	NS
IMD rank, median (IQR)	1 (2)	1 (2)	0.8129	NS
Length of stay (days), median (IQR)	0.329 (1.27)	0.267 (1.81)	0.2495	NS
% length of stay less than 24 hours (n)	67.4 (2113)	61.8 (698)		
% length of stay 24-48 hours (n)	20.6 (646)	14.1 (159)		
% length of stay of 2-5 days (n)	10.9 (343)	19.0 (215)		
% length of stay of 5 days or over (n)	1.02 (32)	5.05 (57)		
% requiring critical care admission (n)	1.34 (30)	3.72 (42)	<0.0001	S
% requiring oxygen (n)	30.3 (949)	28.5 (322)	0.2677	NS
% with at least one respiratory readmission within 12 months (n)	69.2 (2170)	70.0 (790)	0.6467	NS

Table 4.2 – Demographics and Clinical Outcomes of Children aged 2-16 years admitted with VIW or asthma, by age band. S = significant, NS = not significant.

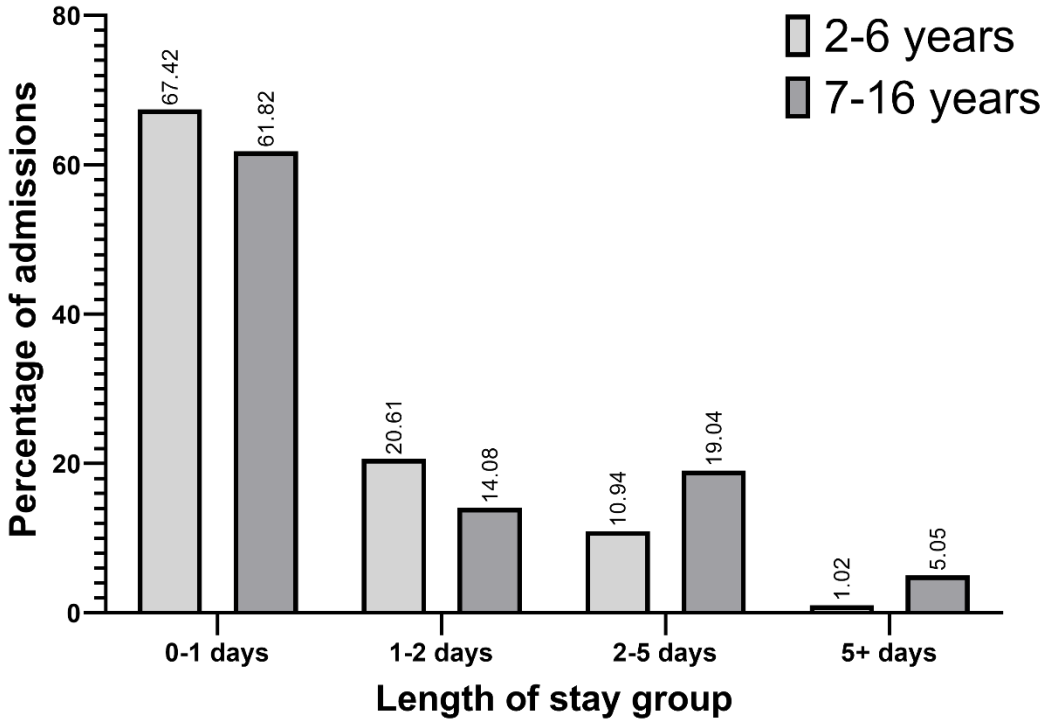


Figure 4.11 – Percentage of admissions in each age band with each length of stay.

4.3.6 Relationship of IMD2019 to admissions and patient outcomes

The English IMD 2019 ranked 32844 small areas, known as Lower-layer Super Output Areas (LSOAs) from 1 (most deprived) to 32844 (least deprived)[294]. The ranks of the postcodes for the home addresses of the children admitted in this period ranged from 10 to 31393, with a median rank of 1822. The IMD also uses deciles to categorise the overall level of deprivation in a LSOA, ranging from 1 (most deprived) to 10 (least deprived). The median IMD 2019 decile of all admissions was 1 (IQR = 2). There were 2466 (57.8%) admissions of children who lived in an IMD 1 decile area, whilst only 8 admissions (0.187%) of admissions were in children from IMD decile 10 postcodes. This distribution is not dissimilar from that for Liverpool as a whole, except for decile 1 (see **Figure 4.12).** For instance, out of the 298 LSOAs in Liverpool, 145 (48.7%) are in the 1st IMD decile and 1 (0.336%) is in the 10th decile[310].

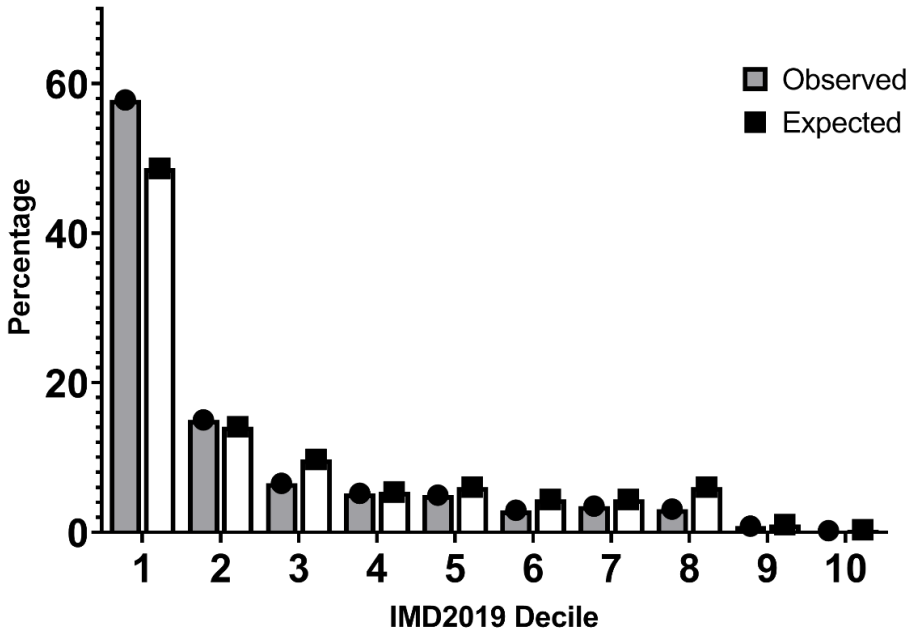


Figure 4.12 – The percentage of admissions from each IMD 2019 decile. These percentages (left bar for each decile) are directly compared to the expected percentage. The expected percentage was calculated by dividing the number of LSOAs in Liverpool in each decile by the total number of LSOAs in Liverpool[310]

The patients were grouped according to their IMD 2019 decile. The groups chosen were deciles 1-3, 4-7 and 8-10, as these are roughly even as proportions of the English population. It was found that there were 3384 admissions in IMD deciles 1 to 3 (2147 male and 1237 female), 705 in IMD deciles 4 to 7 (457 male and 248 female) and 174 admissions from IMD deciles 8 to 10 (135 male and 39 female), see **Figure 4.13.**

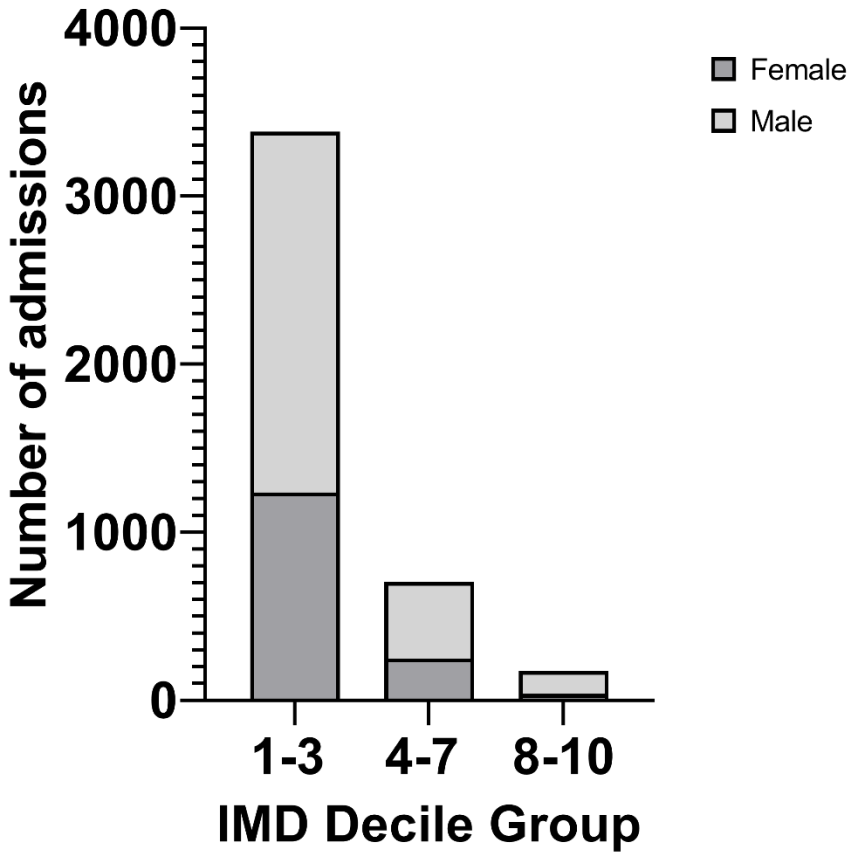


Figure 4.13 – Total admissions grouped according to IMD Decile, using postcode at time of admission, presented in sex categories.

The clinical outcomes of the children in each IMD decile were analysed. The length of stay was significantly different between the deciles ($p<0.0001$) and the IMD groups (deciles 1-3, 4-7 and 8-10), $p=0.0003$. The median length of stay for the 8-10 decile group was moderately shorter than the other two groups (see **Figure 4.14)**. When admissions were split according to length of stay, where observation was defined as less than 6 hours and admission anything longer than this, it was seen that the low deprivation group (IMD 8-10)

was more likely to be observed than admitted, unlike the other two IMD groups. The oxygen requirement appeared to be relatively similar for all IMD deciles, except for the low deprivation group (IMD deciles 8-10), see **Figure 4.16.** It was difficult to conclusively compare the critical care requirement between the deciles as only 72 children were admitted overall**.** However, the overall pattern showed that children from areas of medium to high deprivation (IMD deciles 1-7) were more likely to be admitted to critical care than those from areas with low deprivation. Furthermore, no children admitted from deciles 8-10 required critical care admission. The percentage of patients readmitted with a respiratory diagnosis within a year of admission was not significantly related to the IMD decile.

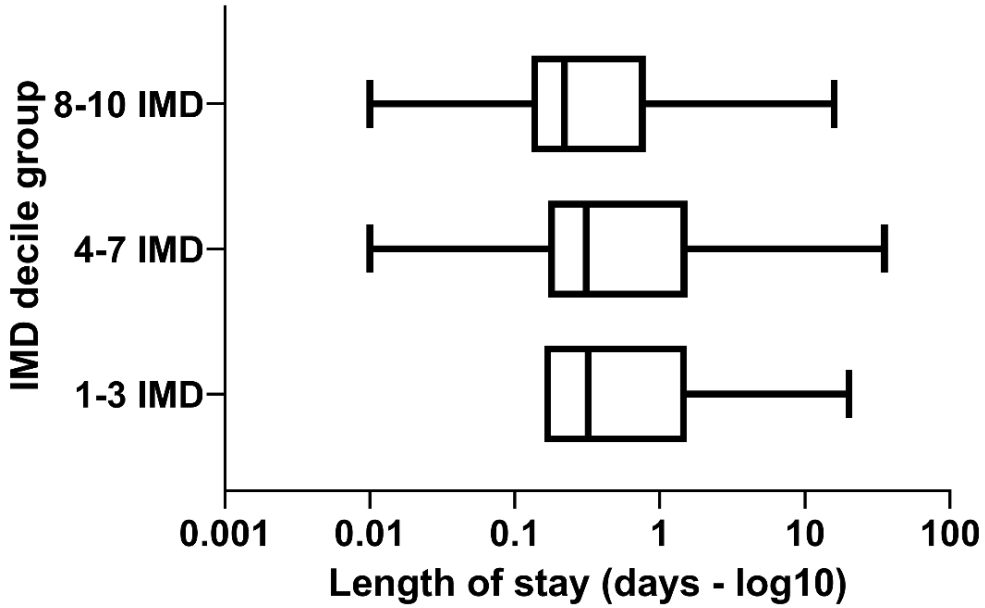


Figure 4.14 – Box plot showing the relationship of IMD decile group and length of stay in days. The scale is logarithmic, using a base of 10.

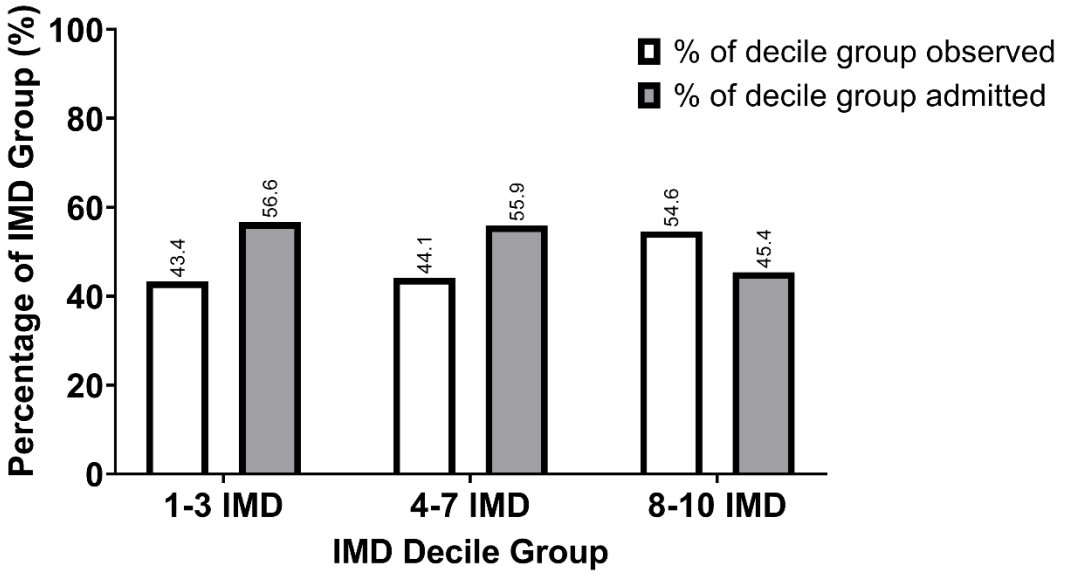


Figure 4.15 – Percentage of each IMD decile group that required observation and admission. Observation was defined as a period of less than 6 hours (0.25 days) between admission and discharge, whereas admission was all remaining lengths of stay.

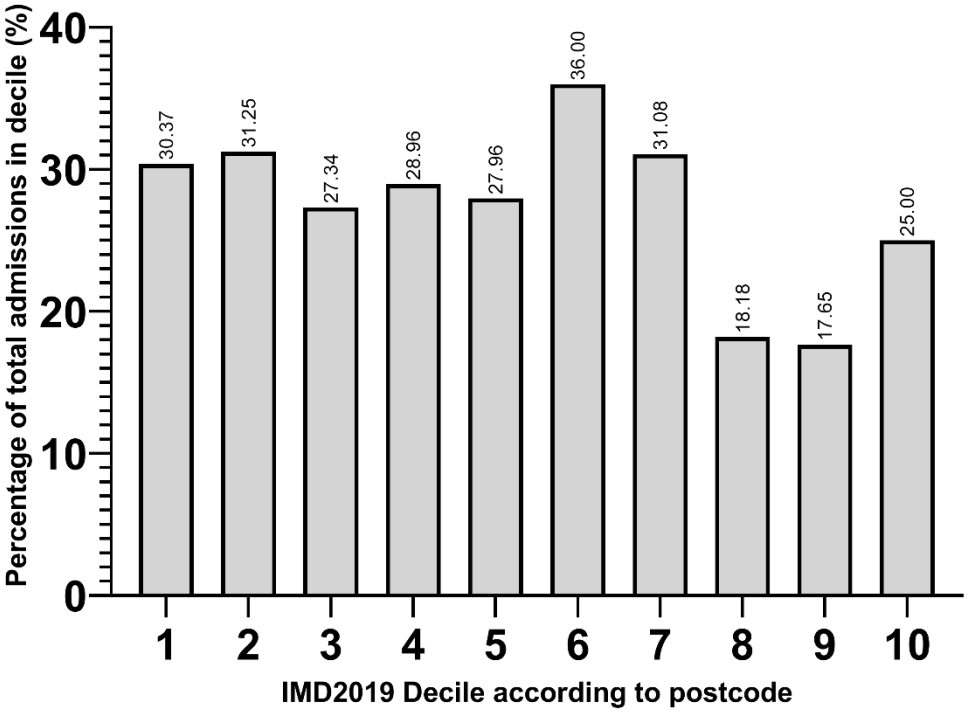


Figure 4.16 – The percentage of total admissions from each IMD decile that required oxygen therapy at some point during their patient stay.

4.3.7 Relationship of air quality to admissions and patient outcomes

At the time of this analysis, NO_2 concentrations were only available up until December 2019 from Liverpool City Council, contained in the Annual Status Reports for Air Quality. Also, some of the diffusion tube data was incomplete. Some of the reasons for this were compromised or missing diffusion tubes, due to vandalism or environmental damage, meaning that no meaningful readings could be made for the specified month. Patients with missing data, who were admitted after December 2019 or who live over 1km from a PDT site, were excluded from the air quality analysis as it was decided that the accuracy of the readings would be reduced due to significant fall off in NO_2 concentrations with distance. Monthly average NO_2 readings were obtained for the date of admission were obtained for the relevant admissions. Nitrogen dioxide emissions data was available for 2423 admissions.

The PDT sites were used to produce Voronoi cells. Each PDT had an 'area of influence', with a maximum distance of one kilometre between the PDT and the outer edge. The postcodes of patients that resided within each of these cells were then ascribed an NO_2 reading using the closest PDT station, for the month in which the admission occurred.

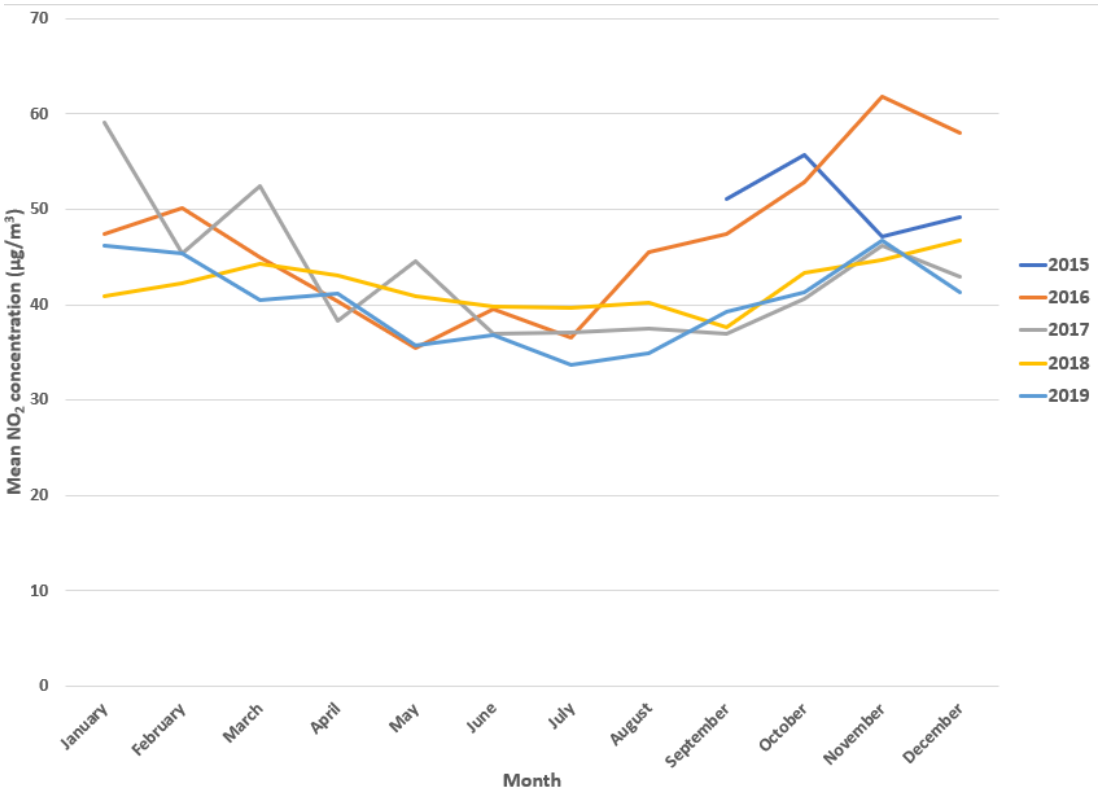


Figure 4.17 – The mean NO_2 concentration for each of the admissions in each calendar month by year

Month	January	February	March	April	May	June
Average monthly NO_2 (µg/m^3)	48.4	45.8	45.5	40.7	39.1	38.3
Month	July	August	September	October	November	December
Average monthly NO_2 (µg/m^3)	36.8	39.5	42.5	46.7	49.4	47.6

Table 4.3 - The mean NO_2 concentration for each month from September 2015-December 2019, including all available data for admissions in the respective month.

The mean nitrogen dioxide concentration for the admissions in each calendar month are plotted in **Figure 4.17**. The overall mean for each month in the period 2015-2019 is presented in **Table 4.3**. This data shows that the average monthly NO_2 concentration for admissions is highest in the Autumn and Winter period, peaking in November at 49.4µg/m^3. The lowest levels of average NO_2 occurred during the Summer, with the lowest concentration occurring in July (36.8µg/m^3).

The mode NO_2 reading (to the nearest whole number) was 41µg/m^3, which was estimated for 104 admissions. The mean estimated nitrogen oxide exposure was 44.0µg/m^3. It was found that 12.3% (297) admissions had exposure to NO_2 levels less than 30µg/m^3 and 25.6% (621) had exposure between 30 and 40µg/m^3. The current WHO recommended limit of 40µg/m^3 was exceeded by 58.7% (1423) of admissions included in this analysis. Within this group, 33.6% (813) had exposure between 40 and 50µg/m^3, 16.3% (394) had exposure between 50 and 60µg/m^3, and 12.3% (298) had exposure over 60µg/m^3.

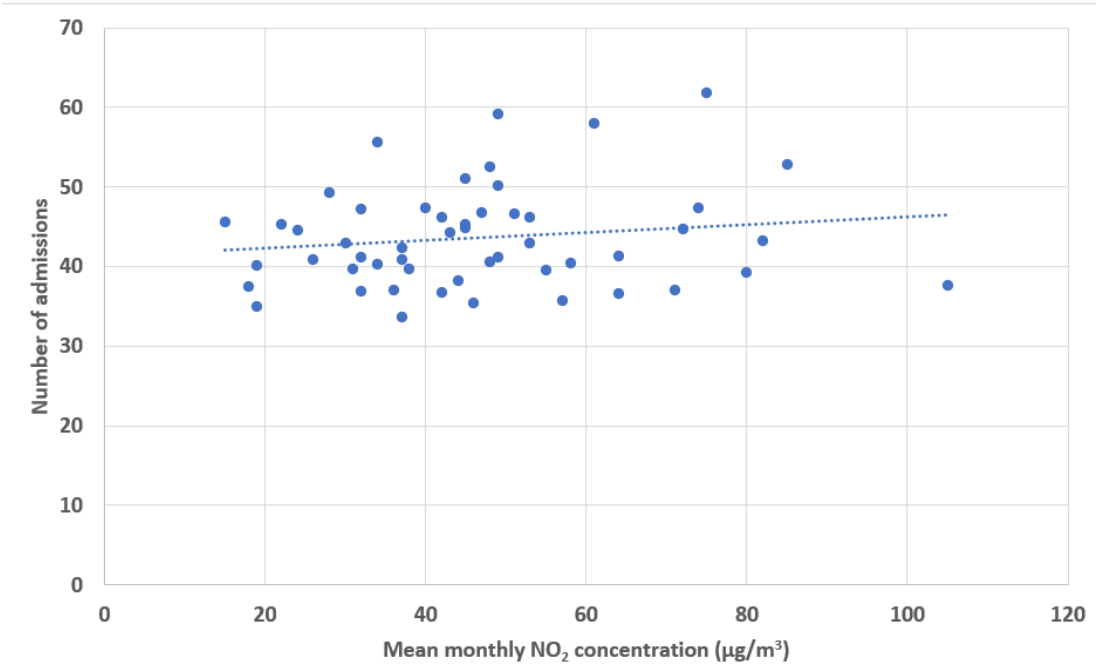


Figure 4.18 – The relationship between the mean nitrogen dioxide concentration for each calendar month of data collection and the associated number of admissions

Figure 4.18 demonstrates a very weak positive correlation between the mean monthly NO_2 concentration and the number of admissions for which air quality data was available. This relationship is not statistically significant (Spearman's correlation coefficient – 0.137, p = 0.334 CI -0.150 to 0.402). Analysis of the clinical outcomes of patients (such as length of stay and oxygen requirement) for each NO_2 group was attempted, but unfortunately there was insufficient data available from the AURNs to allow any meaningful conclusions to be made.

4.4 Ethical approval

This study was registered locally with the Clinical Audit Team at Alder Hey Children's Hospital (reference number 6183). Ethical approval was not required due to the anonymisation of patient details and the retrospective collection of data.

Chapter 5: Discussion and conclusions

5.1 Chapter 2 discussion and conclusions

5.1.1 Strengths and limitations

This systematic review has several strengths, the main one being the adherence to a pre-established protocol. Moreover, the search strategy was developed with careful consideration and rigorous inclusion criteria applied. To minimise the risk of relevant studies being omitted because the wrong search terms were used or an error in screening papers, a specialist librarian was consulted when developing the search strategy, and the screening process was carried out by two reviewers independently. Another strength of this study was the thorough quality assessment performed for all included studies.

The fundamental weakness of systematic review is that its quality is inherently reliant on the quality of the existing literature. On the other hand, by its very nature, systematic review can be a useful tool for identifying gaps in the literature and guiding research priorities. Furthermore, as the evidence base is constantly being updated, the search strategy can readily be repeated to ensure it represents all available evidence. The inclusion criteria for this review specified that only new or modified severity scores were eligible. Consequently, later studies to validate existing severity scores have not been included. There is potential for a further review of all existing validity data for all identified severity scores for preschool wheeze.

A problem encountered during this review was the variety of definitions of preschool wheeze, asthma, and bronchiolitis used in children. As a result, to prevent relevant scores being excluded, some severity scores which do not perform well in the preschool age group may have been included. Furthermore, the specified age range was widened to 6 months - 6 years, as some studies of preschool wheeze use a larger age range[11].

5.1.2 Discussion

This systematic review is currently the most comprehensive review of the tools published used to assess acute wheeze in preschool children. It has highlighted that there are many different severity scores (n=89) published for use in acute wheeze in preschool children, but there is not one widely used tool. It was also found that there are significant differences in the signs used to indicate severity in different age groups, as well as the respiratory rate values. There is significant overlap between many of the severity scores, with most of the scores measuring variations of a few core domains. Some of the severity scores, such as the PRAM, CAS and PASS, have undergone more thorough validation for use than other scores, although none of these scores were fully validated upon their first published use[140, 248, 275].

Assessment of wheeze severity in preschool children is vital to guiding acute management, for example, when deciding on whether to admit a child or choosing the dosing interval for bronchodilators[248]. Different scores may be needed depending on the intended use. Thus, one score may be best at predicting admission, whilst another may better predict end points such as fitness for discharge. Moreover, as previously identified, the optimal severity assessment of wheeze in preschool children varies from the assessment of children of other ages. For instance, many factors vary with the age of children including ability to undertake lung function testing, compliance of the chest wall and the likely underlying pathology[311]. Consequently, efforts should be made to consistently use tools developed specifically for preschool children.

It has been repeatedly concluded that the instruments available for the acute assessment of dyspnoeic or wheezing children are not well validated[138, 277, 278, 312]. In addition, many of the scores published for use in this age group have been informally developed[138]. The previous literature reviews of available severity scores available for wheezing in preschool children used clear definitions for each of the domains of validity that should be considered

when developing a score (such as validity, responsiveness and reliability)[138]. When future attempts to validate these severity scores are undertaken, as many of these domains as possible should be measured[138]. This would allow better comparison of the measurement properties of each score and allow the best performing tool to be selected.

When deciding upon an optimal scoring system for a clinical trial, it is important to be consider how long it takes to use the score, and balance this with the sensitivity of the score. The specialist training required for the accurate use of a clinical scoring system also needs to be considered. For example, some parameters, such as oxygen saturation, are simple to measure. In contrast, some parameters, such as pulsus paradoxus or I:E ratio are very difficult to measure, or require experience. Moreover, parameters are highly subjective, such as 'overall impression of severity', and therefore may reduce score reliability. Clinical judgement of severity is often based on experience, so may not be comparable between members of the clinical team. Some suggestions to increase the interobserver reliability of these severity scores include incorporating their use into the medical school curriculum, producing educational videos of children with wheeze of different severity levels, and incorporating a smaller number of items[140, 313]. However simplification of the score can also limit the utility and its discriminatory power.

Several scores have been derived from the Pulmonary Index[196]. This was first described for use in status asthmaticus by Pierson *et al*. in 1974[196], although it is based on pre-existing scores, including a bronchiolitis score published in 1966[208]. The four key aspects measured by this score are: respiratory rate, wheezing score, I:E ratio and accessory muscle use[196]. The original bronchiolitis score was inspired by a score for respiratory distress syndrome in infants[314], first published in 1956 by Silverman and Andersen. More recently, the Pulmonary Index has been further modified, in 1990[265], 1993[257] and 2005[315]. Variations of

the Pulmonary Index are widely used in children of all ages with acute respiratory distress, particularly in bronchiolitis and asthma[208, 257, 265, 315].

As proposed in a 2004 review of severity scores for preschool asthma, there remains a need for research directly comparing severity scores[138]. There have been few direct comparisons of available instruments. One published in 2016, compared five acute asthma severity scores in children aged 2-16 (Johnson *et al.)*[316]. The selected tools were compared in terms of their interrater reliability and predictive ability for hospital admission using 48 children in a paediatric ED[316]. Scoring instruments were evaluated for inter-rater reliability between staff members and their utility for predicting hospitalization[316]. The five scores assessed included the three most validated scores identified by this review: the PRAM[202], the PASS[222] and Clinical Asthma Score[248]. This study concluded that it is feasible to directly compare multiple severity scores in clinical practice, but further studies with greater numbers of participants were needed to make more robust comparisons[316].

Another study published in 2016 (Eggink *et al.)* directly compared five dyspnoea severity scores, in a sample of 27 children aged 0-8 years[317]. Four of the five scores compared were eligible for inclusion in this systematic review: namely the Asthma Score (AS)[251], PRAM[202], Asthma Severity Score (ASS)[207] and Clinical Asthma Evaluation Score 2 (CAES-2)[226]. All had some weaknesses, with all demonstrating inadequate agreement, concurrent validity and lack of internal consistency[317]. The PRAM[202] and AS[251] showed the greatest validity for use, with satisfactory face and construct validity, ease of use, intra-observer reliability, and floor and ceiling effects[317]. However, this study was limited as it used video recordings and so severity scores requiring auscultation could not be compared[317].

Given the large number of existing scores, new severity scores should not be developed without clear justification. Instead, future studies should report the raw data collected from each patient. This data would facilitate direct comparison of several scores in large

patient cohorts and thus better validation of the existing scores[316, 317]. A previous review of the severity scores for school-age asthma showed that data collection at some hospitals was insufficient to derive the scores identified by the authors[139]. It is likely that this is also true for scores for preschool children.

Moving forward, improved routine data collection using electronic medical records could allow many of the parameters from the scores mentioned in this chapter to be collected, and analysed alongside clinical outcomes[318]. Standardising clinical assessment would require little additional effort but would improve the ability to compare initial severity and treatment responses between settings and over time. Machine learning algorithms could then be used to calculate multiple severity scores and to identify which signs and symptoms best predict outcomes and therefore which scores are most useful or even lead to the development of a new score[319, 320]. Alternatively, clinicians with expertise in assessing and managing children with preschool wheeze could be surveyed to find the domains that would be, in their experience, most important to include in a severity score.

5.1.3 Implications for clinical practice

One of the central messages of this chapter was that new versions of severity scores should not be developed, as there already too many different variations of a few core domains. Instead, efforts should be focussed on validating the existing scores and performing direct comparison of their measurement properties, including validity (face, criterion and construct), responsiveness, reliability and discriminatory power. Ideally, a well-validated severity score developed specifically for preschool wheeze should be used routinely and all measured parameters should be reported in publications, to allow comparison of findings between studies. Additionally, there may be a need for different scores to be used for different settings, such as outpatient and inpatient, as well as for different end points, including the need for admission or ICU.

5.1.4 Implications for future research

Chapter 2 highlighted an urgent need for direct comparison of the severity scores used in preschool children with acute wheeze. Moreover, efforts must be made to undertake further validation of severity scores, especially in relation to their sensitivity and specificity for predicting end points. Further to this, it would be beneficial to survey medical professionals on their preferences when using a scoring system and consider their opinions when assessing which tools are most appropriate for each clinical setting. It would also be useful to measure other aspects of practicability, such as time taken to complete the score.

5.1.5 Conclusion

This chapter summarises the published severity scores used to assess acute wheeze in preschool children, and their validity for use. In conclusion, there have been at least 89 severity scores published for use in acute wheeze in preschool children. Few of these scores have been well validated for use and many of the scores have significant overlap in the parameters used. Further to this, the use of some of the scores published may not be feasible in some clinical environments or without specific expertise. Therefore, it is important for a standardised severity assessment tool specifically developed for use in preschool children to be used routinely and validated, thus improving the utility and transferability of future research results.

5.2 Chapter 3 discussion and conclusions

5.2.1 Limitations

There were some notable limitations in these experiments. The major limitation is pipetting error, which is likely due to my inexperience in the laboratory. Whilst I made efforts to improve the accuracy and reliability of my pipetting, the results do not appear to be consistent and there were several undetermined results, thus limiting the conclusions that could be made. In addition, there were some more minor limitations, such as possible

contamination with DNA as RNase-free DNase was not used to treat the samples[321,322]. Moreover, RNA integrity was not measured before further processing was undertaken.

There was a small sample size of three independent experiments for each time point. For some conditions, there were undetermined results which further reduced the amount of data available. As a result of this limited number of experiments, the conclusions that can be drawn from this work are minimal. If one of the PCR results was inaccurate, this could skew the mean and standard deviation for this time point. This could be overcome in future research by using a greater number of samples for each time point, such as n = 5. Moreover, further time points could have been used to better characterise the time course, for instance at hourly intervals. However, this would have required more cells to be cultured, requiring more time and resources.

Furthermore, cytokine and receptor expression were only measured for 24 hours following viral infection, this could have been extended to identify trends over a longer time course. The same MOI was used for all experiments, which may not have been the optimal dose. As well as this, an ELISA could have been used to measure cytokines in the supernatant[323]. In addition, flow cytometry could have been used to measure cellular surface expression of ICAM-1 and CDHR3[74]. A cytotoxicity assay could also have been used to assess whether the cytokine response was reduced by cell death.

5.2.2 Discussion

These experiments aimed to investigate the expression of ICAM-1, CDHR3 and IL-6 in BEAS-2B cells, following HRV infection using qPCR. In summary, this work shows that although mean IL-6, ICAM-1 and CDHR3 expression increase in the 24 hour period following HRV infection, none of these results are statistically significant.

I did not identify any previous studies comparing receptor expression of the selected respiratory cell lines (A549 and BEAS-2B). It is useful to know that the expression of these

receptors at baseline is not significantly different, as when considered alongside other factors, such as growth requirements and similarity to primary cells, this may help guide the cell line selection for future studies. If it were found that one of the cell lines had significantly higher expression of a receptor, this may have made it the preferred choice for viral infection, as it would be easier to identify changes from the baseline.

Due to the small sample size and large variation in the results, it is not possible to definitively characterise the expression of ICAM-1 and IL-6 following HRV-A infection of BEAS-2B cells. Previous literature has shown that when BEAS-2B cells are infected with HRV 14 and 16, the expression of ICAM-1 is modestly increased[87, 324]. In addition, interesting research using primary human tracheal, nasal and bronchial epithelium has shown that HRV-infection increases ICAM-1 expression following infection, up to approximately 12 times[74, 325,326].

As well as a HRV receptor, ICAM-1 functions as the ligand for leukocyte function-associated antigen 1 (LFA-1), which is found on many cell lineages, including leukocytes[327]. It is believed that the increased expression of ICAM-1 by respiratory epithelium, following HRV infection, facilitates the relocation toward the airway, and activation, of immune cells[91, 280]. This influx of immune cells and associated inflammatory mediators can cause an environment of atopic airway inflammation[280]. There remains a need for more complex studies investigating this relationship in primary cells and in vivo[*280*].

I was unable to identify any previous measurement of CDHR3 expression following HRV-C infection of BEAS-2B cells. Unfortunately, only results up to four hours post-infection were available for analysis. As a result, it is not possible to conclude how CDHR3 expression changes following HRV-C infection using these results. It should be noted that a 2017 study of ciliated primary bronchial epithelial cells, which showed that infection with HRV-C15 is correlated with decreased expression of CDHR3[282].

Regrettably, due to the large degree of variation in the results, I was not able to conclusively characterise the pattern of IL-6 release follow HRV-A and C infection. A study undertaken by Subauste and colleagues showed that 24 hours following infection of BEAS-2Bs with HRV-14, supernatant IL-6 concentration was significantly increased[87]. It has been suggested that this relationship is due to activation of a NF-kB-independent pathway[285]. Moreover, a study of HRV infection (HRV-2 and HRV-14) of primary human tracheal epithelium has shown that this infection is associated with increased production of several cytokines, including IL-6 and IL-8[325].

There was a moderate positive correlation identified between the expression of IL-6 and ICAM-1 in BEAS-2B cells infected with HRV-A. A weak positive correlation was observed between IL-6 and CDHR3 following HRV-C infection, although not at all time points were available for analysis. No previous work detailing these relationships was identified, suggesting that this may be an area of future research.

5.2.3 Implications for future research

The work reported in chapter 3 was an interesting experiment, which is highly relevant to the pathophysiology of preschool wheeze, more specifically EVW. Regrettably, the scope of this work was limited by time, experience and resource constraints. In order to expand on these findings, future research should investigate the production of more cytokines and inflammatory mediators in relation to more subtypes of HRV infection. It would also have been insightful to expand the time course, perhaps to 72 hours and use more samples at each time point. Additionally, other methods of quantification of these receptors and cytokines could be undertaken, such as ELISA. Whilst the BEAS-2B cell line is a reasonably faithful model of bronchial epithelium, it would be interesting to compare the results of this work with those found using primary bronchial epithelial cells, especially pBECs collected from children with preschool wheeze, as this would be a more representative model of the in vivo environment[328].

5.2.4 Conclusions

In conclusion, preliminary results using the BEAS-2B cell line suggest that HRV-A infection is associated with a small increase in mean IL-6 and ICAM-1 expression in the 24 hours following infection, although none of these results were statistically significant. Additionally, infection with the more recently discovered subtype HRV-C was associated with similar minor increases in mean IL-6 and CDHR3 expression, however again these were insignificant. There was a moderate positive correlation observed between mean IL-6 and ICAM-1 expression following HRV-A infection. The conclusions are significantly limited by the small sample size and large degree of variation of the results. Further research using both respiratory cell lines and primary cells is required to definitively elucidate these complex relationships, and investigate the underpinning immunological pathways.

5.3 Chapter 4 discussion and conclusions

5.3.1 Strengths and limitations

The main strength of this study is the large population size and extensive data set, including demographics and a range of clinical outcomes, such as length of stay, oxygen requirement, critical care requirement and number of readmissions. Moreover, this dataset includes a wide age range of children, from 2-16 years, and allows comparison between preschool and school-aged children. In addition, this data makes use of physician-coded diagnoses, therefore the bias of self-reported wheezing is minimised.

The classification of childhood wheezing illness is imprecise and presents a diagnostic challenge, especially in preschool children. This is due to overlap of presentations, conflicting definitions, and lack of reliable diagnostic testing[1, 3]. As a result, a range of OPCS codes were included in this analysis, to ensure that relevant admissions were not excluded.

The relatively long period of data collection is advantageous as it allows any repeat admissions to be identified and allows yearly patterns in admissions to be described. It also

means that a period of abnormal admissions (such as during the COVID-19 pandemic) has only a minor effect on the entire dataset. Liverpool is an interesting city to study as it has relatively high levels of both deprivation and air pollution. Moreover, the fact that much of the city is served by a single children's hospital (AHCH) means that the admission rates should be roughly representative of the overall number of cases of viral-induced wheeze and asthma in this large urban area. It is a particularly exciting time to explore the trends in childhood respiratory health, as Liverpool commences on its journey to become a UNICEF Child Friendly city[290]. This means that the starting point for acute respiratory admission rates can be identified, and any improvements over time measured.

Another strength of this study was the careful consideration of the methods used. The codes used to identify the patients were refined multiple times to ensure that they were fit for purpose. Multiagency working between AHCH and Liverpool City Council allowed a broad set of relevant data to be identified. Furthermore, the statistical tests were tailored to the variable types and presence of normality.

This study does have a number of limitations. For instance, the data was hospital-based rather than population-based and does not include children treated in primary care or outpatient clinics, so the patterns observed may not be representative of the whole population. It is also unknown which of the children had received community treatment before their admission, which may have affected the course of their illness. In addition, there may have been other factors at play which had an influence on the likelihood of admission, such as the provision of community ambulatory care or the occupancy rates of hospital beds at AHCH, which were not measured in this dataset.

Furthermore, this data was collected from a single large urban hospital in a deprived area of England, therefore the findings may not be transferable to other settings. Moreover, due to the retrospective nature of the data collection, there may be some information bias in

the results as it is reliant on the accuracy of the coding system. Some of the diagnosis codes may not be correct, leading to inaccuracies in the number of identified admissions.

In addition, the Index of Multiple Deprivation is frequently updated to ensure its accuracy and to track changes over time. The most recent versions are the IMD 2019 and 2015. The IMD 2019 used throughout this study for consistency, however this may not have been fully representative for the years before 2019. Data for the whole years of 2015 and 2020 was not collected as the data was collected between 1st September 2015 and 31st August 2020. As a result, some months have data for 2015-2019 whereas other months have data for 2016-2020. This data may not be fully comparable due to changes over time, especially with the possible influence of the COVID-19 pandemic and its associated social distancing measures in 2020[329, 330]. Data from the first wave of COVID-19 in 2020, collected from a multinational cohort of children with asthma, have shown better control and reduced frequency of exacerbations[331]. This is also likely to be true of viral-induced wheeze, and has been attributed to reduced exposure to triggers, including circulating viral infections[331]. Moreover, due the lockdown, traffic reduced significantly, and air quality improved[332].

The data collected does not include the viral species responsible for exacerbations of wheeze. This may be a confounding variable as children of different ages are more likely to have different infections[271, 333]. Moreover, viral infections can vary in severity according to aetiology, for instance rhinovirus C infections can often be more severe than other infections[78].

Northern England has a temperate climate, with extremes of temperature rarely occurring. As a result, these results may not be transferable to other settings with different climate types, as there is some evidence linking extreme temperatures to increased emergency department admissions[334]. Moreover, all children in the UK should have access to healthcare free at the point of use, thanks to the National Health Service (NHS). Therefore,

these results may not be transferable to other settings, where private health insurance is required, as financial cost may be a factor in the decision to present to medical services.

The ethnic group for each individual was not included in the dataset, meaning that differences between groups could not be adjusted for. A previous population-based study in Leicestershire, UK showed that there are higher rates of admissions for wheeze or asthma in South Asian children aged 2-4 years than in white children[335]. Moreover, analysis of data collected by the UK Millennium Cohort study revealed significant differences between ethnic groups, in terms of the prevalence of asthma and wheezing symptoms[336]. Whilst many of these differences were accounted for socioeconomic factors or cultural practices, these differences highlight the importance of considering and adjusting for ethnicity where appropriate[336]. It would also be insightful to explore the data to see how the admissions and clinical outcomes of children and families who do not speak English compare to those fluent in English language, as language barriers have previously been suggested as a factor in inequalities in healthcare accessibility for acute asthma[337].

The home addresses of each patient at time of admission were used, so some patients who recently moved to the area and thus had limited exposure to the local levels of air pollution could have been included. This is a minor limitation, as short-term temporal trends in air pollution are believed to have a greater impact than long-term levels on acute admissions[338, 339]. Moreover, some patients may have moved away from the Liverpool area during the period of data collection, so data for subsequent admissions may not be available. In addition, some children may have been transferred to adult services during the study period or been admitted to other local hospitals, such as Arrowe Park Hospital, on the Wirral.

Data collected reflected outdoor air quality, which may not be representative of the air quality in the homes of patients[340]. The group level measures of postcode and Index of

Multiple Deprivation were used to measure individual exposure to air pollution and deprivation. The overall trends shown by these figures may not be representative on an individual level, so called ecological fallacy.

Moreover, the environmental tobacco smoke exposure of each patient was not available as part of this dataset. This is a notable omission as it has recently been demonstrated by Mackay *et al.* that in Scotland there has been a significant reduction in the number of children aged 0-5 years requiring hospitalisation for severe wheezing illness since the introduction of a smoking ban in cars in December 2016[341]. This result suggests that cigarette smoke exposure is an important factor in the admission rates of children with preschool wheeze[341], perhaps more so than outdoor air pollution, which could have been adjusted for. Moreover, the data for other important air pollutants, such as particulate matter, was not available[342].

5.3.2 Main findings

This study represents a detailed review of the demographic features and clinical outcomes of children admitted with VIW or asthma to a single specialist Children's hospital in Liverpool. This large data set spanning the whole age range from 2-16 years provides an interesting insight into annual trends in admissions and the relationship between demographics, clinical outcomes and social characteristics.

This study provided some valuable data regarding the temporal trends of admissions, including month, day and time. This information is very useful when planning a clinical trial for asthma or VIW, as it allows efficient resource allocation. For instance, more research nurses may be recruited to work at busy times, such as the Autumn and Winter months.

It was also shown that significantly more male children are admitted than females. There were some significant differences between the sexes identified, with female patients more likely to have a longer length of stay than their male peers, and more likely to require

oxygen. On the other hand, male patients were more likely to be admitted to critical care. The average age of female patients was significantly greater than male patients at the time of admission. This study demonstrated that the majority of admissions (73.5%) for VIW and asthma are in the 2-6 years age band, with a distribution skewed towards the lower end of this band. Risk factors, including age and sex, could potentially be used to generate a risk tool to help predict admission or other clinical outcomes.

This medical record review also revealed important trends in length of stay, oxygen requirement, critical care admission and readmissions. The majority of patients had a short patient stay of less than 24 hours and only 2.09% had a length of stay greater than 5 days. According to the medical record data, 29.8% of admissions required oxygen at some point during admission. Only 72 of the admissions required admission to critical care. Of note, older patients (7-16 years) are significantly more likely to require critical care admission than preschool children. The length of stay of those admitted to critical care was significantly longer than the other patients. Moreover, this analysis showed that there is a very high rate of readmission with respiratory diagnoses within 12 months at 69.4%.

The final key area of this study was the exploration of two social characteristics: deprivation and air pollution exposure. It was shown that the majority of admissions to AHCH with VIW and asthma are from the most deprived 10% of the national population (IMD decile 1), although this proportion is skewed in Liverpool. In addition, the average length of stay of these patients is significantly longer than that of the least deprived. No children from the least deprived areas (IMD 8-10) were admitted to critical care. It was found that there was a non-significant weak positive correlation between mean monthly NO_2 concentration and the number of associated admissions. This suggests that there are other more significant factors influencing admission rates. It was found that nearly 60% of

admissions had an estimated mean monthly NO_2 exposure exceeding the WHO air quality guidelines[117].

5.3.3 Findings in context of other studies

The findings presented here are an important addition to the evidence base, much of which was published over a decade ago, and is specific to asthma only[31, 343, 344]. A striking difference in the number of admissions between males and females was observed, with significantly more admissions in males of both age bands. This is supported by the findings of previous studies, including two UK birth cohort studies, which found an excess prevalence of males with asthma and wheeze before puberty, compared to females[345, 346]. Another cohort study of children aged 2-13 years presenting to ED in the USA and Canada found a similar proportion of males (61%)[347]. The most widely accepted explanation for this sex difference in pre-adolescent children is that females have comparatively larger bronchial radius than males relative to their size[347]. Moreover, it is thought that there are mechanical differences in the lungs of males and females, with females able to increase their maximal flow, possibly through dilation of the airways[348].

The majority of children admitted were less than 6 years of age, and the median age at admission was 4.16 years. This result is similar to the findings of a UK national audit undertaken by Davies *et al.* for the period 1998-2005, which found the majority of acute wheeze/asthma admissions were aged less than 5 (median 3 years)[31]. In addition, a multicentre prospective study of 1578 children aged 1-14 by Hilliard *et al.* found that 62% of paediatric acute asthma admissions were aged less than 5 years[343].

Hilliard and colleagues also examined the temporal trends in paediatric acute asthma admissions[343]. The pattern of monthly admissions was very similar to that reported here, with September being the most common month, followed by November, with much lower admission rates over the Spring and Summer months[343]. However, the Hilliard study stated

that no weekly pattern in admissions was observed, whereas the results presented here showed a significant variation in admissions between days[343]. Hilliard *et al.* also examined the diurnal variation in acute asthma admissions. The pattern observed was similar, with 57% of admissions between 5pm and 9am, compared to 61.5% in this work[343].

The median length of stay was 1 day, which is in concordance with the findings of other similar studies[31]. It was found that nearly 30% of admissions required oxygen at some point during admission, determined using the Paediatric Early Warning Score (PEWS). This figure is moderately less than what has been reported in similar patient cohorts, with a frequency of approximately 40%[31]. It is possible that this figure may not be fully representative due to inaccuracies in the use of the PEWS score. Moreover, the distinction between oxygen and forms of non-invasive ventilation was not made, which may have been more insightful. The percentage of patients admitted who required critical care admission was very similar to the findings of a previous retrospective study of asthmatic children aged 0-18 years in Toronto (1.69% at AHCH vs. 1.78%)[344]. However, it must be noted that this data was from 1983-1992, so it may be out of date as clinical practice has evolved significantly[344].

Rates of readmission for acute asthma varies widely between countries. For instance, the incidence of readmission varies from 40% in Oulu, Finland[349] to 15% in Rhode Island, USA[350]. A previous UK audit found that 34% of children admitted with asthma had also been admitted in the preceding 12 months[31]. Analysis of the readmissions in Rhode Island revealed that children living in neighbourhoods with higher proportions of ethnic minority residents and poverty were more likely to be readmitted[350]. The high rates of respiratory readmission within 12 months of initial admission (69.4%) show a potential for improvement, for instance through improved community follow-up or more comprehensive action plans. More tailored treatment according to the phenotype and/or

endotype may also be beneficial[351]. However it should be noted that these readmissions included all coded respiratory diagnoses, not only VIW and asthma.

Statistically significant differences in terms of clinical outcomes, including length of stay, oxygen requirement and readmission within 12 months, were observed between sexes. This is in disagreement with two previous studies, which included children with asthma, which suggested that exacerbation severity is independent of sex[347, 352]. In future analyses, the data should be adjusted for confounding factors, such as age, air pollution exposure, deprivation and cigarette smoke exposure, to ensure all differences observed are attributable to sex.

It was shown that children with a postcode in an IMD decile 1 area were more likely to be admitted with an exacerbation of VIW or asthma than any other children. Meanwhile, recent analysis of over 200000 medical records of patients with asthma was used alongside the Welsh IMD to explore whether there is inequality in asthma care in Wales, according to levels of deprivation[353]. It showed that there were 8.2% more primary care visits per patient for asthma-related issues in the most deprived areas compared to the least deprived areas[353].

It was found that there was a very weak positive correlation between NO_2 exposure and admission numbers. The characterisation of the relationship between air pollution and admissions is limited as other air pollutants, such as particulate matter, may be more significant[342]. This weak relationship suggests that other factors are more significant in influencing admission rates, these may include the patterns of viral circulation and school or day care attendance[354].

A recent study undertaken by the Environmental Research Group at King's College London, in collaboration with the British Lung Foundation, concluded that up to 1,040 deaths a year can be linked to the air pollutants NO_2 and $PM_{2.5}$ in the Liverpool City Region[355, 356]. It was

also found that an eight year old child currently residing in the Liverpool City Region could have their life expectancy reduced by up to five months by air pollution, even if pollutant concentrations are reduced to meet future targets[355, 356]. Further analysis of this data revealed that on 'high pollution' days in Liverpool an additional seven children aged 0-14 years are admitted to hospital with asthma and 12 children with asthma experience worsening of symptoms, such as dyspnoea or cough[355, 356].

Bradford, like Liverpool, is a city with high levels of deprivation and air pollution. A recent environmental study collected data in Bradford to estimate the number of asthma cases in children per year linked to NO_2 and NO_x exposure, as well as those cases associated with traffic emissions[357]. It was estimated 18-38% of all asthma cases in children in Bradford may be due to exposure to outdoor air pollution (NO_2 and NO_x)[357]. Further analysis of the data showed that up to 24% all paediatric asthma cases in Bradford may be due to traffic emissions[357]. These findings are supported by a Danish cohort study, which showed that NO_2 and NO_x are significantly correlated with the development of wheeze in children aged less than 3 years[358]. Whilst these results are not fully transferable, this suggests that many cases of paediatric wheezing illness could be prevented by reducing air pollution.

As well as the impact of air pollution on the incidence of wheezing illness[359], pollutants have been linked to worse asthma control and increased exacerbation frequency and severity[360]. For example, a study of children with asthma aged 6-17 years showed that the further each child lived from a major road, the better their long-term asthma control[360]. Furthermore, a study of London primary schools analysed environmental and health data, finding that an average of 82 asthma exacerbations per school could be prevented if outdoor nitrogen dioxide levels were reduced[361]. In addition, a study of over 46000 children in Hong Kong showed that nitrogen dioxide concentration is significantly associated ($r=0.63$, $p=0.028$) with the number of hospitalisations for acute preschool wheeze[362]. A

potential mechanism for exacerbation caused by nitrogen dioxide is the generation of reactive nitrogen species, leading the epithelial injury[363].

The mechanisms linking air pollution and asthma have not been fully elucidated but it is thought that pollutant particles can damage lung tissue by producing reactive oxygen species, or alternatively by stimulating inflammation[364]. Whilst air pollution can affect the health of all ages, young children are particularly vulnerable as their lungs are still developing, air pollution exposure can have a greater effect on lung function[364]. For example it was shown that childhood exposure to air pollution can reduce the lung growth of children by 4.6% by age 15 years, which may lead to suboptimal lung function throughout adulthood[355].

5.3.4 Implications for clinical practice

There are three main clinical implications of chapter 4. Firstly, it was revealed that AHCH has a high rate of readmissions in children admitted with VIW and asthma. This suggests that there is potential for better community follow up[365]. Moreover, this may also hint at some flaws in the development of, or adherence to, action plans[31, 365]. Clinicians may also consider other measures to reduce readmission, including parental education and ensuring adherence to preventer medications[365]. It may be useful to perform further investigation of factors responsible for this relatively high proportion of readmissions.

Secondly, this chapter provided significant insight into the issue of air pollution in Liverpool. In clinical practice, it may be possible to improve the education of clinicians, and therefore patients, about the adverse effects of air pollution[304]. Utilising this improved knowledge, clinicians may be able to provide patients with strategies to help reduce their exposure to air pollution, such as regularly monitoring air quality, walking away from main roads when possible and avoiding going outside during a rush hour[342]. Technology may be helpful in delivering up-to-date pollution data to individuals, especially through websites and apps.

One example is the British Lung Foundation website, which gives health advice relating to local air pollution[301]. However, these measures only have a limited impact, and should be combined with wider societal efforts to reduce air pollution[342].

Thirdly, this chapter highlighted the significant impact of deprivation on the number of children admitted with VIW and asthma. Whilst healthcare professionals are limited in their power, they are provided with an often unparalleled position of trust in patients' lives. As such, clinicians often become aware of the social situation of patients. Therefore, clinicians may be able to give targeted support and signposting of resources to those in the most deprived sections of society[366].

At Alder Hey Children's Hospital there not is currently a protocol for which children with preschool wheeze should be followed up in the community. There is also not a standard education programme for parents and children and no consensus on which children benefit most from intervention. It may also be beneficial to introduce a standard timeline for follow-up, to ensure that high risk children receive crucial interventions. The high rate of readmission in this study suggests that there is scope for improvement in follow up.

5.3.5 Implications for government and society

The wider implications of this thesis mainly relate to the findings of chapter 4. These can be classified into two groups: socioeconomic deprivation and air pollution. Firstly, when considering deprivation, it has been demonstrated that children from more disadvantaged areas are more likely to have poor respiratory health in childhood. The factors for this are yet to be fully explored, and potential confounders should be considered. However, this result suggests that whilst more should be done to promote healthy lifestyles and good community management of all children, these efforts should be targeted on those most vulnerable to the effects of deprivation. There exists a wide disparity in paediatric respiratory health between the most and least deprived deciles[367]. This makes the case for

a more fair society, with better support for those most deprived and more of a focus on health promotion, rather than emergency treatment[368]. Some potential strategies to help reduce these inequalities include the promotion of breastfeeding and smoking cessation before pregnancy[116].

Additionally, the results of the air quality analysis highlight a critical need to improve air quality in Liverpool. These figures are likely to be similar in other cities across the UK. Efforts from policymakers and the public should focus on reducing air pollution, for example by promoting public transport, green energy and encouraging walking and cycling[355]. The current government plans to ban the sale of petrol and diesel fuelled cars from 2030 should significantly reduce air pollution[342]. However, by this stage another generation of children will have been adversely affected. It has been argued that improving air quality, and the associated respiratory morbidity, should be given higher political priority[342]. So far, only 17 out of 62 local authorities have planned to establish a clean air zone, which illustrates the potential for much greater positive change[301].

5.3.6 Implications for future research

The retrospective medical record analysis, as well as the combination of air quality and socioeconomic deprivation data, reported in chapter 4 has many potential research implications. Whilst these findings from Liverpool provided an insight into the admissions patterns for VIW and asthma at AHCH, they may not be transferable to other settings. As such, future research should compare admissions to multiple different hospitals and settings, across the UK. As part of this, it would be useful to compare the primary care and ED attendances with those patients who require hospital admission. International collaboration could allow VIW and/or asthma admissions to be compared between countries, and the associated demographic and social factors to be explored.

Another interesting avenue of research would be investigating the effects of COVID-19 on the number of admissions and clinical outcomes of children with VIW and asthma. Furthermore, it would be interesting to compare admissions pre-COVID-19, during lockdown and following the relaxation of COVID-19 restrictions, to analyse any difference in admissions and clinical outcomes. Further to this, comparing the Liverpool air quality data pre- and post-COVID-19 would be insightful. However, it would be difficult to differentiate whether any changes in admission rates were due to lockdown and social distancing or improved air quality.

The findings of this work make a convincing case for more research into the effects of deprivation and air pollution on childhood respiratory health, especially to track improvements associated with interventions. Further to this, there should be more research into air pollution, to find out which interventions have the greatest impact. Some potential options, which could be trialled, include clean air zones in urban areas, legislation to discourage pollution and localised interventions, such as reducing traffic flow near schools[342, 355]. Furthermore, additional research using more localised air pollution data, including home monitoring, could be used to allow a more accurate measurement of personal exposure to air pollutants[369]. Additionally, there should also be more research into the mechanisms underlying the association between socioeconomic deprivation and poor childhood respiratory health, especially episodic viral wheeze[367]. Whilst there are some proposed mechanisms, more research is needed to clarify this complex interaction, and therefore allow targeted prevention strategies.

A 2013 survey of emergency departments in the UK and Ireland showed that there was a high degree of variation in ED management of acute paediatric wheeze, despite the existence of national guidelines[370]. These findings highlight the need for further research across multiple settings, to better reflect UK clinical practice and account for variations in

the population. One of the vital parts of management of exacerbations of viral wheeze and asthma is compiling a comprehensive action plan and arranging appropriate follow-up, in order to prevent recurrent admissions[371]. Whilst an asthma action plan is routine practice in the UK, it would be interesting to investigate the proportion of readmissions with an action plan and community follow-up, to see if there is scope for improvement. Furthermore, in order to explore the reasons for admission and also possible interventions to prevent readmission, future studies could use qualitative interviews to explore patient experiences more holistically.

5.3.7 Conclusion

In conclusion, VIW and asthma are important causes of acute hospital admission in children. Age and sex are significantly associated with the number of admissions and the clinical outcomes of these children. Length of stay is usually short, and relatively few children require oxygen or critical care admission. The number of respiratory readmissions to AHCH within 12 months is very high. Deprivation is linked to increased numbers of admissions and a high proportion of admitted children had NO_2 exposure exceeding the WHO guidelines. These results provide an important indication of admission patterns at a large specialist Children's hospital. They could be used to inform clinical trial planning, improve interventions to prevent readmission and promote optimal childhood respiratory health, especially through air quality improvement and overcoming the effects of deprivation.

5.4 Synthesis of key themes

Although initially the themes discussed in each chapter may seem unconnected, there are multiple strands that intrinsically link these sections. When considered together, the key results highlight the need for holistic patient management of preschool wheeze, at once considering the severity of an exacerbation, the pathophysiological pathways, and environmental exposures of the patient. If this more comprehensive approach is

incorporated into future patient interactions and clinical trials, it could help to further advance the evidence-base and facilitate more personalised patient care, thus potentially improving outcomes.

Air pollution has been associated with an increased risk of paediatric acute asthma admissions, although different pollutants contribute in different amounts[372]. Additionally, air pollution has been linked new asthma diagnoses, though the available evidence is not conclusive[373]. It should be noted that the effect of NO_2 has been refuted or deemed minimal in some studies[372], but significant in others[374]. It has also been found that air pollution can increase the susceptibility of an individual to viral respiratory infections, which includes viral-induced asthma/wheeze exacerbations[375]. Some of the proposed mechanisms underlying this interaction are: alteration of host anti-viral responses (including macrophages), reduced respiratory epithelial barrier function, and oxidative stress caused by air pollutants[373, 375]. Those with an underlying preschool wheeze or asthma diagnosis are more vulnerable to these insults, due to the existing inflammatory landscape that exists in their lungs[373]. However, there remains a need for more focussed research to elucidate the specific mechanisms of action of each air pollutant and to investigate any genetic variants linked to susceptibility to air pollution-related airway injury[364]. Furthermore, the existing research is largely focussed on asthma in adolescents and adults, so more attention should be paid to preschool wheeze.

In addition, a cohort study of 114 children published in the Lancet aged 8-11 years showed that high levels of personal NO_2 exposure in the week before a viral-induced asthma exacerbation was linked to more severe lower respiratory tract symptoms or reduced peak flow rate[305]. This finding is supported by a laboratory-based study of rhinovirus infection of BEAS-2B cells, which showed a synergistic relationship between HRV infection and exposure to oxidants (NO_2 or ozone), on the subsequent release of proinflammatory

cytokines[49]. When taken together, these findings suggest that air pollution could play an important role in both the incidence and severity of acute childhood wheeze, and provide a possible mechanism.

Another connection between chapters that can be made is the relationship between socioeconomic deprivation and severity of acute wheeze or asthma exacerbations. There has been limited research detailing this relationship, and even less focusing on preschool wheeze. Moreover, no studies using a severity score as the clinical outcome measure for children with different deprivation levels were identified. Studies of asthmatic patients have mostly shown greater severity and worse clinical outcomes in those with lower socioeconomic status[376]. For instance, a recently published Welsh national cohort study that used the Welsh IMD, has shown that compared to the least deprived patients, the most deprived patients had more ED attendances linked to asthma and more acute asthma admissions. Furthermore, the clinical outcomes for these patients were worse, with a longer average length of hospital stay and a higher risk of asthma-related death[376]. However, the median age was 47.5 years and so is unlikely to represent the population affected by preschool wheeze[376]. The paucity of available data and lack of consistent use of a clinical indicator, such as a severity score, show the need for more research into the relationship between deprivation and acute severity of preschool wheeze.

Whilst causality between socioeconomic status and susceptibility to respiratory infections has not been established, there is a strong association between these factors[367]. In particular, this association is strongest for the preschool age group (0-4 years)[367]. An ecological study by Hawker *et al*. found that in the 0-4 years age group, the admission rate was a disturbing 91% higher in the most deprived children compared to the least deprived[367]. The underlying mechanisms have not been fully detailed, but some factors

implicated in this relationship include: increased exposure to respiratory infections, increased exposure to tobacco smoke and poor housing[367, 377].

Another area of contention is the relationship between air pollution, socioeconomic deprivation and acute asthma or wheeze. Importantly, little of this evidence includes children. Some studies have shown that deprivation has no significant impact on the effects of air pollution on asthma exacerbation frequency and reliever medication use[378, 379]. On the other hand, some studies have suggested that socioeconomic status may influence the susceptibility of children to air pollution-related asthma exacerbations[380]. Conversely, a recent Health Impact Assessment study, conducted in Barcelona, suggested that those who are least deprived may be most vulnerable to the effects of air pollution on asthma[381]. Whilst the study design limits the comparability of these results, they demonstrate that more research is needed to fully elucidate the relationship between deprivation, air pollution and respiratory health, especially in children. This is likely to be a complex relationship, with both factors intricately linked. For instance, it should be noted that the most deprived in society are often most at risk from exposure to air pollution[382].

5.5 The future of wheeze in preschool children

An aspect of personalised medicine which is increasingly being adopted in paediatric respiratory care is the use of phenotypes and endotypes[383]. A phenotype is how an individual's genome is expressed in terms of observable characteristics[351]. This may change over time, according to environmental exposures[351]. On the other hand, an endotype is a subgroup of a disease underpinned by a specific pathophysiological process[351]. Some characteristics used to define endotypes of asthma include: response to treatment, lung physiology, clinical presentation, and genetics[351]. A simple distinction between these concepts is that phenotyping often leads to generic treatment strategies, whereas endotyping allows more targeted regimens[383].

The INFANT trial investigated individualised therapy in 300 children aged 1-6 years with persistent asthma[384]. This study suggested that phenotyping with blood eosinophil levels and aeroallergen sensitisation can guide treatment strategies[384]. For instance, this method of phenotyping can identify preschool children at high risk of exacerbation, who may therefore benefit from maintenance ICS therapy[384]. It has also been recommended that spirometry should be measured where possible in this age group[383]. In addition, during wheezing episodes, the response to inhaled bronchodilators should be determined, perhaps using a severity assessment tool[383].

Whilst there have been great advances in the understanding the pathophysiology of wheezing illness in children, one major criticism of much research is its failure to acknowledge the varied endotypes that exist within this syndrome[385]. It is hoped that the distinction of asthma endotypes will allow more targeted treatment, more accurate prognostication, and better understanding of underlying inflammatory pathways, thus improving outcomes[383, 385]. For example, it could advance understanding of the pathophysiology of future risk, including the risk of exacerbations and lung function decline, which could be important in implementing personalised medicine[383].

Another example of personalised medicine is the use of biologics, which are approved for the treatment of severe asthma in children aged 6-18 years[386]. Each biologic acts on a specific target in an inflammatory pathway. For example, omalizumab is a monoclonal anti-IgE antibody that stops unbound IgE from binding to receptors on immune cells[386]. Whilst there are no biologics licensed for use in preschool children, this is a potential area of development[386]. However, these treatments are expensive and only for specialist use[386].

5.5.1 Future clinical and research priorities in preschool wheeze

A 2020 Spotlight article in the Lancet Respiratory Medicine highlighted some areas which should be targeted to facilitate improved quality of care in preschool wheeze[387].

Additionally, the 2014 ERS Task Force Update also provided a list of future research priorities[7]. Features of the proposed integrated clinical model include: augmenting patient and public engagement with improving research and clinical practice, encouraging specialists to work collaboratively, and making healthcare more accessible to children with preschool wheeze[387]. Targets in the realm of quality of life identified include: developing PROMs (see **section 1.1.3**), understanding the effects on caregivers, improving clinical outcomes, and improving patient-doctor exchanges[7, 387]. Another priority area identified is improving understanding of the pathogenesis of preschool wheeze, particularly the factors influencing recurrence, the effect of environmental exposures, acute management of exacerbations, as well as improving acute severity assessment and discharge planning[7, 387]. It was also proposed that there should be more RCTs of therapies for preschool wheeze, including samples with a range of characteristics included, such as atopic sensitisation[7]. Furthermore, researchers should aim to identify improved genetic markers and biomarkers for use in predicting response to treatment, thus allowing more personalised treatment[7]. The work contained in this thesis will contribute to some of these identified priorities, including improving acute severity assessment and understanding the effects of environmental exposures on preschool wheeze.

5.6 Conclusions

Preschool wheeze is a common, and often poorly understood, presentation. There exists a need to improve clinical assessment, immunological understanding and social inequalities associated with admission (including air pollution and deprivation). Together, these results highlight the value of holistic care for preschool wheeze, considering acute severity, pathophysiology, and the patient environment. If this approach is widely implemented in research, policy and clinical practice, it could improve acute management and long-term respiratory outcomes for children. The future of preschool wheeze is exciting, with the

prospect of personalised medicine, optimisation of management and better understanding of the pathophysiology.

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Appendix

Appendix 1 – PROSPERO Systematic Review Protocol (Reference CRD42020212507)

1. Review title.

What severity scores have been published for use in acute wheeze in preschool children?

2. Original language title.

English

3. * Anticipated or actual start date.

06/10/202

4. * Anticipated completion date.

31/03/202

6. Named contact.

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10. * Organisational affiliation of the review.

University of Liverpool

Organisation web address:

https://www.liverpool.ac.uk

11. * Review team members and their organisational affiliations.

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12. * Funding sources/sponsors.

None

13. * Conflicts of interest.

List actual or perceived conflicts of interest (financial or academic).

None ◉

Yes ○

14. Collaborators.

None

15. * Review question.

What severity scores have been published for clinical use in acute wheeze in preschool children and how well have these been validated for use?

16. * Searches.

MEDLINE, Scopus, Web of Science and CINAHL databases will be searched, with no date or language restrictions. The references of all full-text papers eligible for inclusion will be screened for relevant publications using a backward searching strategy. The full search strategy for each database will be saved and the results imported to EndNote for reference management.

17. URL to search strategy.

None

18. * Condition or domain being studied.

Wheeze in preschool children (incorporating preschool wheeze/viral-induced wheeze and asthma).

19. * Participants/population.

Children aged 1-5 years with acute wheeze who presented to a healthcare setting.

To be included studies must include human children aged 1-5 who present acutely with wheezing illness.

Publications will be excluded if they include non-human participants, if data only includes patients aged <1 year or >5 years, if the data for the 1-5 age group cannot be separately extracted or if they include wheeze caused by other conditions such as pneumonia or cystic fibrosis.

20. * Intervention(s), exposure(s).

The intervention is a clinical score to assess the severity of an exacerbation.

To be included each score must include at least two different parameters and a numerical value must be assigned to each parameter, before a composite measurement is made. Scores eligible for inclusion must be evaluative or discriminatory scores used to assess severity of acute wheeze in a clinical setting and they must be applied by a medical professional. Only papers detailing a novel or modified score will be eligible for inclusion.

Scores that assess long term asthma risk (predictive scores), scores that measure quality of life, scores that use special tests (such as blood tests or pulmonary function testing), scores that assess long term symptom control and scores that use historical information such as

medication history or parent-reported questionnaires will be excluded. Scores that involve the measurement of only one parameter or scores with no numerical value assigned to each parameter will be excluded. Publications which do not detail the parameters used in the score and how the overall score is calculated will be excluded.

21. * Comparator(s)/control.

All other relevant severity scores identified during the review and their associated validity data.

22. * Types of study to be included.

All study designs will be included. Systematic reviews, guidelines, protocols and commentaries will be excluded.

23. Context.

Studies measuring severity of acute preschool wheeze in healthcare settings with a new or modified score.

24. * Main outcome(s).

Novel or modified severity scores published for acute wheeze in preschool children.

*** Measures of effect**

Not applicable

25. * Additional outcome(s).

The parameters included in each severity score, the number of severity scores that include each parameter, the number of participants in each study, the details of how to apply each scoring system, the weighting applied to different parameters and the clinical setting (ED/inpatient/community). Other secondary outcomes that will be extracted relating to the methodological quality of the study include: the basis for item selection, validity, responsiveness, reproducibility, discriminatory power, utility for use in preschool children and reliability.

*** Measures of effect**

Please specify the effect measure(s) for you additional outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

Not applicable

26. * Data extraction (selection and coding).

The PRISMA framework will be used to guide this review. Firstly, all results will be deduplicated. Then two reviewers will separately screen the titles of all results, using the inclusion and exclusion criteria. Then all publications eligible for the next stage of screening will have their abstract screened by both reviewers independently. Any disagreements will be overcome by consensus or consultation with a third party if needed. Excel spreadsheets will be used to store all information, including reasons for decisions, and these will be saved at each stage to ensure a clear data trail throughout. Next, all of the papers eligible

for the next stage will have the full-text obtained and then they will be reviewed using the same criteria to assess eligibility for inclusion. Any publications eligible for full-text review will be recorded in a table and the reasons for exclusion will be recorded if appropriate.

One reviewer will extract the relevant data into a predetermined data extraction table using an Excel spreadsheet, which will be checked by the other reviewer. If there are any disagreements, these will be settled by discussion with an independent third party. The data extracted will be the name of the score, the parameters used in each severity score, the number of participants in each study, the setting where the score is used and the details of how to apply each scoring system. The basis for item selection will also be recorded, for example expert opinion, results of previous studies, theoretical models, patient or parent suggestions, clinical observations, existing severity scores or routine data collection. Other secondary outcomes that will be extracted if they are reported include: the basis for item selection, validity (including type of validity: face, criterion and/or construct), responsiveness and reliability (internal consistency and interrater consistency).

27. * Risk of bias (quality) assessment.

The risk of bias will be assessed independently by two researchers, with any disagreements resolved by an independent third party. The appropriate risk of bias tool for each study design type will be used and if necessary help will be sought from a statistician.

28. * Strategy for data synthesis.

The data extracted will be the name of the score, the parameters used in each severity score, the number and age of participants in the first sample, the setting where the score is used (e.g. ED/community/inpatient), the country of origin of the score and the details of how to apply each scoring system (the number and names of parameters, assigned values and attributed scores). The weighting of each of the parameters within the scores will be assessed and compared. For each study, the following outcomes will be recorded if they are reported: the basis for item selection (e.g. expert opinion/previous scores/clinical observations), validity (including type of validity: face, criterion and/or construct), responsiveness, reliability (internal consistency and interrater reliability), reproducibility, utility in young children and discriminatory power. The definitions for these terms will be based on those used in Bekhof *et al*. 2014 (Systematic review: Insufficient validation of clinical scores for the assessment of acute dyspnoea in wheezing children) which assessed dyspnoea scores for the entire paediatric age group. This will allow a methodological assessment of the study in which each score was developed. The data reported in each study will be summarised in a simplified table to allow clinicians to quickly appraise the quality of evidence supporting the use of each severity score.

The components of the severity score will be extracted from each of the included studies into a data extraction table produced using Microsoft Excel. At least two studies will be required for data synthesis. No meta-analysis is planned and no statistical software package will be needed.

29. * Analysis of subgroups or subsets.

Not planned.

30. * Type and method of review.

Type of review

- Service delivery
- Systematic review

Health area of the review

- Child health
- Respiratory disorders

31. Language.

- English

There is an English language summary.

There is not an English language summary

32. * Country.

- England

33. Other registration details.

None

34. Reference and/or URL for published protocol.

None

35. Dissemination plans.

Do you intend to publish the review on completion?

Yes

No

Give brief details of plans for communicating review findings.?

A paper will be submitted to a journal specific to this field.

36. Keywords.

Preschool wheeze, viral-induced wheeze, preschool asthma, severity score, clinical score, children, human, acute, exacerbation

37. Details of any existing review of the same topic by the same authors.

None

38. * Current review status.

Ongoing

39. Any additional information.

This record has been amended to ensure that it meets the required standards for a systematic review of methodology. The methodology proposed for this review is similar to that of Hawcutt *et al*. (Paediatric acute asthma scoring systems: a systematic review), which was successfully registered in 2018 and published in JACEP Open in June 2020.

40. Details of final report/publication(s) or preprints if available.

No

Appendix 2 – PRISMA checklist for systematic reviews[174]

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	37
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	N/A
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	37
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	37
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	37
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	39

Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	38
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	**Appendix 2**
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	39-41
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	41
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	39-40
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	42
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	39
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	N/A
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	42
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A

RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	40-41
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	43
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	**Appendix 5, 6, 7 and 8**
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	N/A
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	42-60
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	60-61
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research. Q2	61-65

FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

Appendix 3 – Search terms used (the same for each database)

Keyword: Wheeze

Search Terms used: wheez* OR asthma

Keyword: Severity score

Search Terms used: “severity scor*” OR “pulmonary ind*” OR “pulmonary scor*”

Keyword: Child

Search Terms used: preschool* OR pre-

school* OR child* OR p?diatric* OR infant* OR toddler*

Keyword: Acute

Search Terms used: acute OR emergenc* OR exacerbation*

Appendix 4 – Reasons for exclusion of full text articles

Title	Author and Year	Reason for exclusion
The RAD score: a simple acute asthma severity score compares favorably to more complex scores[388]	Arnold *et al*. 2011	Does not meet age criteria
Noninvasive Testing of Lung Function and Inflammation in Pediatric Patients with Acute Asthma Exacerbations[389]	Arnold *et al*. 2012	Does not meet age criteria
The Pulmonary Index: Assessment of a Clinical Score for Asthma[390]	Becker *et al*. 1984	Does not meet age criteria
Large observer variation of clinical assessment of dyspnoeic wheezing children[136]	Bekhof *et al*. 2015	No composite measurement
Comparison of intravenous terbutaline versus normal saline in pediatric patients on continuous high-dose nebulized albuterol for status asthmaticus[197]	Bogie *et al*. 2007	Does not meet clinical setting criteria (ICU)

Severe Respiratory Syncytial Virus Infection in Hospitalized Children Less Than 3 Years of Age in a Temperate and Tropical Climate[391]	Butler *et al*. 2019	Not an acute clinical assessment (retrospective)
Bacteremia in Children Hospitalized with Respiratory Syncytial Virus Infection[392]	Cebey Lopez *et al*. 2016	Not an acute clinical assessment (retrospective)
Comparison of salbutamol efficacy in children--via the metered-dose inhaler (MDI) with Volumatic spacer and via the dry powder inhaler, Easyhaler, with the nebulizer--in mild to moderate asthma exacerbation: a multicenter, randomized study[393]	Direkwatanachai *et al*. 2011	Does not meet age criteria
An Index Predicting Relapse and Need for Hospitalization in Patients with Acute Bronchial Asthma[394]	Fischl *et al*. 1981	Does not meet age criteria
Diagnostic value and pathophysiologic basis of pulsus paradoxus in infants and children with respiratory disease[395]	Frey *et al*. 2001	Not an acute clinical assessment (requires blood gas)
The value of pulsus paradoxus in assessing the child with status asthmaticus[396]	Galant *et al*. 1976	Does not meet clinical setting criteria (ICU)
Corticosteroids not recommended for viral wheeze in ages 10 to 60 months[397]	Gilbert *et al*. 2009	No full text
Global strategy for asthma management and prevention[398]	GINA 2015	No new/modified severity score (grades only)
Prednisolone Plus Albuterol Versus Albuterol Alone in Mild to Moderate Bronchiolitis[399]	Goebel *et al*. 2000	Does not meet age criteria
Helium-oxygen improves Clinical Asthma Scores in children with acute bronchiolitis[400]	Hollman *et al*. 1998	Does not meet clinical setting criteria (ICU)
Asthma severity at night during recovery from an acute asthmatic attack[401]	Hoskyns *et al*. 1991	Does not meet age criteria
Does nebulized hypertonic saline shorten hospitalization in young children with acute viral wheezing?[402]	Kanjapradap *et al*. 2018	No new/modified severity score

Clinical-physiologic correlations in acute asthma of childhood[403]	Kerem *et al*. 1991	Does not meet age criteria
Heliox Therapy in Infants With Acute Bronchiolitis[404]	Martinon-Torres *et al*. 2002	Does not meet clinical setting criteria (ICU)
Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants[405]	Midulla *et al*. 2010	Does not meet age criteria
Acute effect of nebulized budesonide in asthmatic children[406]	Nuhoglu *et al*. 2005	Does not meet age criteria
Association of rhinovirus infection with increased disease severity in acute bronchiolitis[126]	Papadopoulos *et al*. 2002	Does not meet age criteria
Association of various weight-based doses of continuous albuterol on hospital length of stay[407]	Parlar-Chun *et al*. 2020	Cannot extract scoring system
Emergency department use of ketamine in pediatric status asthmaticus[408]	Petrillo *et al*. 2001	Does not meet age criteria
Influenza and other respiratory viruses: standardizing disease severity in surveillance and clinical trials[409]	Rath *et al*. 2017	Does not meet clinical setting criteria (ICU)
Bordetella pertussis infection attenuates clinical course of acute bronchiolitis[410]	Raya *et al*. 2013	Does not meet age criteria
Effect of oral glucocorticoid treatment on serum inflammatory markers in acute asthma[411]	Sahid El-Radhi *et al*. 2000	Does not meet age criteria
Efficacy of frequent nebulized ipratropium bromide added to frequent high-dose albuterol therapy in severe childhood asthma[412]	Schuh *et al*. 1995	Does not meet age criteria
Management of acute asthma in childhood. A randomized evaluation of beta-adrenergic agents[413]	Schwartz *et al*. 1980	Does not meet age criteria
Early predictors of admission or prolonged emergency department treatment for children with acute asthma[414]	Shope *et al*. 2001	No new/modified severity score
A controlled clinical trial of effects of water mist on obstructive respiratory signs, death rate and necropsy findings among premature infants[314]	Silverman *et al*. 1953	Does not meet age criteria

Comparison of the Therapeutic Effects of Salbutamol Nebulize with different Concentrations of Saline on Children with Bronchiolitis[415]	Soleimani *et al*. 2020	Cannot extract scoring system
Viral etiology of acute lower respiratory tract infections in hospitalized young children in Northern Taiwan[416]	Sung *et al*. 2011	Not an acute clinical assessment (retrospective)
Dexamethasone and Salbutamol in the Treatment of Acute Wheezing in Infants[417]	Tal *et al*. 1983	Does not meet age criteria
Aerosolized budesonide in asthmatic infants: A double blind study[418]	Van Bever *et al*. 1990	Not an acute clinical assessment
Effectiveness and safety of inhaled corticosteroids in controlling acute asthma attacks in children who were treated in the emergency department: A controlled comparative study with oral prednisolone[419]	Volovitz *et al*. 1998	Does not meet age criteria
A multicenter, randomized, double-blind, controlled trial of nebulized epinephrine in infants with acute bronchiolitis[420]	Wainwright *et al*. 2003	Does not meet age criteria
Oral corticosteroids for wheezing attacks under 18 months[421]	Webb *et al*. 1986	Not an acute clinical assessment (parental score)
Transcutaneous oxygen and carbon dioxide levels and a clinical symptom scale for monitoring the acute asthmatic state in infants and young children[422]	Wennergren *et al*. 1986	No new/modified severity score (grades only)
A Critical Asthma Standardized Clinical and Management Plan Reduces Duration of Critical Asthma Therapy[423]	Wong *et al*. 2017	Does not meet clinical setting criteria (critical care)
β2-Adrenergic receptor promoter haplotype influences the severity of acute viral respiratory tract infection during infancy: A prospective cohort study[424]	Wu *et al*. 2015	Does not meet age criteria

Appendix 5 - Full list of parameters included in each severity score

Key: light blue = specific to preschool age group (6 months-6 years), dark blue = general paediatric severity scores (above and below 1-5 age group), yellow = scores developed in preschool age group (1-5 years) and above and purple = scores developed in the preschool age group (1-5 years) and below.

Overall signs observed	Subtype of sign	Alario *et al.* 1992	Arroyo *et al.* 2020	Bajaj *et al.* 2006	Bamberger *et al.* 2012	Bentur *et al.* 1990	Bentur *et al.* 1992	Berger *et al.* 1998	Bohé *et al.* 2004	Can *et al.* 1998	Carit*g et al.* 1999	Chong *et al.* 2017	Coarasa *et al.* 2010	Coarasa *et al.* 2010	Constantopoulos *et al.* 2002	Dabbous *et al.* 1966	Dabbous *et al.* 1966	Daugbierg *et al.* 1993	De Boeck *et al.* 1997	Deerojanawong *et al.* 1994	Eiaz *et al.* 2015	Gajdos *et al.* 2009	Gern *et al.* 2002	Giugno *et al.* 2004	Groothuis *et al.* 1993	Groothuis *et al.* 1990	Jartti *et al.* 2006	Lai *et al.* 2004	Levy *et al.* 2004	Lowell *et al.* 1987	Macias *et al.* 2015
Respiratory rate	**Respiratory rate/tachypnea**			■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■		■	■		■
Respiratory support	**O2 saturation/hypoxia**		■		■						■				■				■					■	■	■			■		
	Supplemental oxygen										■																				
	Assisted ventilation																								■	■					
Auscultation	**Wheeze/rhonchi**		■													■	■	■		■			■		■			■	■		■
	Wheeze location									■																				■	
	Inspiratory wheeze	■		■		■	■	■	■			■	■	■	■				■	■	■						■				
	Partial inspiratory wheeze	■								■																				■	
	Full inspiratory wheeze									■														■						■	
	Inspiratory breath sounds										■																				
	Unequal inspiratory breath sounds																														
	Decreased/absent inspiratory breath sounds																														
	Expiratory wheeze	■		■		■	■	■	■		■	■	■	■	■					■	■						■				
	Mild expiratory wheeze			■																											

Overall signs observed	Subtype of sign	Alario *et al.* 1992	Arroyo *et al.* 2020	Baiai *et al.* 2006	Bamberger *et al.* 2012	Bentur *et al.* 1990	Bentur *et al.* 1992	Berger *et al.* 1998	Bohé *et al.* 2004	Can *et al.* 1998	Caritg *et al.* 1999	Chong *et al.* 2017	Coarasa *et al.* 2010	Coarasa *et al.* 2010	Constantopoulos *et al.* 2002	Dabbous *et al.* 1966	Dabbous *et al.* 1966	Daugbierg *et al.* 1993	De Boeck *et al.* 1997	Deerojanawong *et al.* 1994	Elaz *et al.* 2015	Gaidos *et al.* 2009	Gern *et al.* 2002	Giugno *et al.* 2004	Groothuis *et al.* 1993	Groothuis *et al.* 1990	Jartti *et al.* 2006	Lai *et al.* 2004	Levy *et al.* 2004	Lowell *et al.* 1987	Macias *et al.* 2015
	Moderate expiratory wheeze																													
	Severe/marked expiratory wheeze																													
	Full/pan expiratory wheeze						■			■									■			■		■						■	
	End expiratory wheeze	■				■	■	■		■		■	■	■					■	■	■	■		■						■	
	Crackles/rales										■														■						
	Adventitial sounds																								■	■					
	Air entry/air exchange/breath sounds										■										■										
	Reduced/poor air entry										■									■											■
	Absent air entry/inaudible/silent chest					■		■	■																					
	Aeration (fields)			■																										
	Audible wheeze (without stethoscope)	■				■		■		■			■	■	■	■	■		■			■		■			■				
I:E ratio	Inspiration to expiration ratio															■	■										■				
	Prolongation of expiration (I<E)																	■													
Grunting	General grunting				■											■	■														
	Mild/infrequent	■																													
	Moderate/intermittent	■																													
	Marked/persistent	■																													
	Severe/persistent	■																													
Apnoea	Apnoea																						■	■							
Inspection	General accessory muscle use/retractions			■		■	■	■	■				■	■				■	■			■	■		■	■		■			■

Overall signs observed	Subtype of sign	Alario *et al.* 1992	Arroyo *et al.* 2020	Baiai *et al.* 2006	Bamberger *et al.* 2012	Bentur *et al.* 1990	Bentur *et al.* 1992	Berger *et al.* 1998	Bohé *et al.* 2004	Can *et al.* 1998	Caritg *et al.* 1999	Chong *et al.* 2017	Coarasa *et al.* 2010	Coarasa *et al.* 2010	Constantopoulos *et al.* 2002	Dabbous *et al.* 1966	Dabbous *et al.* 1966	Daugbierg *et al.* 1993	De Boeck *et al.* 1997	Deeroianawong *et al.* 1994	Elaz *et al.* 2015	Gaidos *et al.* 2009	Gern *et al.* 2002	Giugno *et al.* 2004	Groothuis *et al.* 1993	Groothuis *et al.* 1990	Jartti *et al.* 2006	Lai *et al.* 2004	Levy *et al.* 2004	Lowell *et al.* 1987	Macias *et al.* 2015
	Intercostal							■				■	■	■							■	■						■				
	Superior intercostal										■																					
	Inferior intercostal										■																					
	Intercostal (mild/moderate)	■			■					■		■																			■	
	Intercostal (marked)	■			■					■																					■	
	Subcostal		■		■						■	■								■	■	■										
	Subcostal (mild/moderate)	■								■		■																			■	
	Subcostal (marked)	■								■																					■	
	Supraclavicular excavation										■										■											
	Supraclavicular (mild/moderate)									■																					■	
	Supraclavicular (marked)									■																					■	
	Upper chest retraction															■	■															
	Lower chest retraction															■	■															
	Tracheal tug																															
	Abdominal breathing																											■				
	Thoracoabdominal paradox																															
	Suprasternal/tracheosternal				■	■		■			■	■								■	■										
	Sternocleidomastoid																															
	Scalene																															
	Substernal/xiphoid retraction															■	■				■	■										

Overall signs observed	Subtype of sign	Alario *et al.* 1992	Arroyo *et al.* 2020	Baiai *et al.* 2006	Bamberger *et al.* 2012	Bentur *et al.* 1990	Bentur *et al.* 1992	Berger *et al.* 1998	Bohé *et al.* 2004	Can *et al.* 1998	Caritg *et al.* 1999	Chong *et al.* 2017	Coarasa *et al.* 2010	Coarasa *et al.* 2010	Constantopoulos *et al.* 2002	Dabbous *et al.* 1966	Dabbous *et al.* 1966	Daugbierg *et al.* 1993	De Boeck *et al.* 1997	Deerojanawong *et al.* 1994	Elaz *et al.* 2015	Gaidos *et al.* 2009	Gern *et al.* 2002	Giugno *et al.* 2004	Groothuis *et al.* 1993	Groothuis *et al.* 1990	Jartti *et al.* 2006	Lai *et al.* 2004	Levy *et al.* 2004	Lowell *et al.* 1987	Macias *et al.* 2015
	Head bobbing											■									■											
Dyspnoea	General dyspnoea/breathlessness						■														■						■					
	Orthopnoea																															
	Speech impairment																				■											
Nasal flaring	General nasal flaring				■	■		■	■	■	■	■	■	■		■	■			■	■	■					■					
	Mild/infrequent	■																														
	Moderate/intermittent	■																														
	Marked/persistent	■																														
	Severe/persistent	■																														
Colour/cyanosis	Colour (pale/mottled)	■																														
	Cyanosis																						■					■				
	Circumoral/perioral cyanosis	■								■				■																		
	Cyanosis at rest	■								■				■		■																
	Cyanosis with crying/on exertion	■								■				■		■																
	Generalised cyanosis	■								■				■		■																
	Central cyanosis																															
	Peripheral cyanosis															■																
	Mild cyanosis														■	■																
	Moderate cyanosis														■																	
	Severe cyanosis														■																	

Overall signs observed	Subtype of sign	Alario *et al.* 1992	Arroyo *et al.* 2020	Baiai *et al.* 2006	Bamberger *et al.* 2012	Bentur *et al.* 1990	Bentur *et al.* 1992	Berger *et al.* 1998	Bohé *et al.* 2004	Can *et al.* 1998	Caritg *et al.* 1999	Chong *et al.* 2017	Coarasa *et al.* 2010	Coarasa *et al.* 2010	Constantopoulos *et al.* 2002	Dabbous *et al.* 1966	Dabbous *et al.* 1966	Daugbierg *et al.* 1993	De Boeck *et al.* 1997	Deerojanawong *et al.* 1994	Eiaz *et al.* 2015	Gaidos *et al.* 2009	Gern *et al.* 2002	Giugno *et al.* 2004	Groothuis *et al.* 1993	Groothuis *et al.* 1990	Jartti *et al.* 2006	Lai *et al.* 2004	Levy *et al.* 2004	Lowell *et al.* 1987	Macias *et al.* 2015
Behaviour	**Mental status/cerebral function**											■																			
	Awareness/conciousness																														
	Drowsy/confused											■																			
	Coma/obtunded																														
	Distress	■																													
	Anxiety																														
	Activity															■				■											
	Agitated/depressed											■									■										
Cardiovascular signs	**Heart rate/pulse**					■	■		■		■		■		■														■		
	Pulsus paradoxus																														
Signs of infection	**Cough**																														
	Cough on stimulus															■															
	Spontaneous cough															■															
	Occasional cough															■															
	Paroxysmal/frequent cough				■											■															
	Mild cough																							■							
	Moderate cough																							■							
	Severe cough																							■							
	Fever																							■							
	Rhinorrhoea				■																										

Overall signs observed	Subtype of sign	Alario *et al.* 1992	Arroyo *et al.* 2020	Baiai *et al.* 2006	Bamberger *et al.* 2012	Bentur *et al.* 1990	Bentur *et al.* 1992	Berger *et al.* 1998	Bohé *et al.* 2004	Can *et al.* 1998	Caritg *et al.* 1999	Chong *et al.* 2017	Coarasa *et al.* 2010	Coarasa *et al.* 2010	Constantopoulos *et al.* 2002	Dabbous *et al.* 1966	Dabbous *et al.* 1966	Daugbierg *et al.* 1993	De Boeck *et al.* 1997	Deerojanawong *et al.* 1994	Elaz *et al.* 2015	Gaidos *et al.* 2009	Gern *et al.* 2002	Giugno *et al.* 2004	Groothuis *et al.* 1993	Groothuis *et al.* 1990	Jartti *et al.* 2006	Lai *et al.* 2004	Levy *et al.* 2004	Lowell *et al.* 1987	Macias *et al.* 2015
	Mild rhinorrhoea																						■							
	Moderate to severe rhinorrhoea																						■							
	Airway secretions				■																									
	Hoarseness																						■							
Feeding/dehydration	Feeding - eating and drinking														■						■									
	Appetite																													
	Dehydration																													
Palpation	Resonance															■														
	Hyperinflation																													
	Liver and spleen															■	■													
Demographics	Age of child																													
	Duration of illness																						■							
	Weight of child																													
Overall severity	General impression of severity/general condition																	■										■		

Overall signs observed	Subtype of sign	McCallum *et al.* 2013	Meiias *et al.* 2013	Obata *et al.* 1992	Ochoa Sangrador *et al.* 2012	Pancham *et al.* 2016	Pavón *et al.* 1999	Ralston *et al.* 2010	Reed *et al.* 2012	Rivera-Sepulveda *et al.* 2021	Schuh *et al.* 1990	Walsh *et al.* 2004	Wang *et al.* 1992	McCallum *et al.* 2013	Meiias *et al.* 2013	Ater *et al.* 2012	Bano *et al.* 2018	Chalut *et al.* 2000	Parkin *et al.* 1996	Tal *et al.* 1990	Connett *et al.* 1993	Davis *et al.* 1977	Dev *et al.* i 1997	DiGiulio *et al.* 1993	Ducharme *et al.* 1997	Freelander *et al.* 1984	Giordano *et al.* 2012	Gorelick *et al.* 2004	Hambleton *et al.* 1979	Hurwitz *et al.* 1984	Kamps *et al.* 2014
Respiratory rate	**Respiratory rate/tachypnea**	■	■	■		■	■	■		■		■	■		■	■	■		■	■	■		■	■		■	■				■
Respiratory support	**O2 saturation/hypoxia**	■	■						■	■						■	■	■													■
	Supplemental oxygen					■																									■
	Assisted ventilation																														
Auscultation	**Wheeze/rhonchi**		■	■					■			■									■	■	■			■			■		
	Wheeze location																				■										
	Inspiratory wheeze	■					■	■		■					■	■	■	■	■	■				■	■		■				
	Partial inspiratory wheeze																														
	Full inspiratory wheeze										■																				
	Inspiratory breath sounds												■																		
	Unequal inspiratory breath sounds																													■	
	Decreased/absent inspiratory breath sounds																													■	
	Expiratory wheeze						■						■			■	■	■	■	■					■		■				
	Mild expiratory wheeze																											■			
	Moderate expiratory wheeze																											■		■	
	Severe/marked expiratory wheeze																											■		■	
	Full/pan expiratory wheeze	■						■		■	■				■									■							
	End expiratory wheeze	■					■	■		■	■		■		■	■	■			■				■							
	Crackles/rales				■																								■		
	Adventitial sounds																														

Overall signs observed	Subtype of sign	McCallum *et al.* 2013	Meiias *et al.* 2013	Obata *et al.* 1992	Ochoa Sangrador *et al.* 2012	Pancham *et al.* 2016	Pavón *et al.* 1999	Ralston *et al.* 2010	Reed *et al.* 2012	Rivera-Sepulveda *et al.* 2021	Schuh *et al.* 1990	Walsh *et al.* 2004	Wang *et al.* 1992	McCallum *et al.* 2013	Meiias *et al.* 2013	Ater *et al.* 2012	Bano *et al.* 2018	Chalut *et al.* 2000	Parkin *et al.* 1996	Tal *et al.* 1990	Connett *et al.* 1993	Davis *et al.* 1977	Dev *et al.* i 1997	DiGiulio *et al.* 1993	Ducharme *et al.* 1997	Freelander *et al.* 1984	Giordano *et al.* 2012	Gorelick *et al.* 2004	Hambleton *et al.* 1979	Hurwitz *et al.* 1984	Kamps *et al.* 2014
	Air entry/air exchange/breath sounds									■						■	■														
	Reduced/poor air entry							■					■					■			■				■				■		
	Absent air entry/inaudible/silent chest				■													■		■					■			■	■		
	Aeration (fields)							■										■							■						
	Audible wheeze (without stethoscope)	■			■		■				■	■	■		■			■		■					■		■				
I:E ratio	Inspiration to expiration ratio																		■	■				■							
	Prolongation of expiration (I<E)																											■			
Grunting	General grunting																														
	Mild/infrequent																														
	Moderate/intermittent																														
	Marked/persistent																														
	Severe/persistent																														
Apnoea	Apnoea																														
Inspection	General accessory muscle use/retractions		■	■		■	■	■	■				■	■						■	■	■	■	■	■	■	■	■		■	
	Intercostal									■		■			■	■	■		■					■				■		■	
	Superior intercostal																														
	Inferior intercostal																														
	Intercostal (mild/moderate)	■									■																				
	Intercostal (marked)	■									■										■										
	Subcostal									■		■	■						■					■					■		■

Overall signs observed	Subtype of sign	McCallum *et al.* 2013	Meiias *et al.* 2013	Obata *et al.* 1992	Ochoa Sangrador *et al.* 2012	Pancham *et al.* 2016	Pavón *et al.* 1999	Ralston *et al.* 2010	Reed *et al.* 2012	Rivera-Sepulveda *et al.* 2021	Schuh *et al.* 1990	Walsh *et al.* 2004	Wang *et al.* 1992	McCallum *et al.* 2013	Meiias *et al.* 2013	Ater *et al.* 2012	Bano *et al.* 2018	Chalut *et al.* 2000	Parkin *et al.* 1996	Tal *et al.* 1990	Connett *et al.* 1993	Davis *et al.* 1977	Dev *et al.* i 1997	DiGiulio *et al.* 1993	Ducharme *et al.* 1997	Freelander *et al.* 1984	Giordano *et al.* 2012	Gorelick *et al.* 2004	Hambleton *et al.* 1979	Hurwitz *et al.* 1984	Kamps *et al.* 2014
	Subcostal (mild/moderate)																				■										
	Subcostal (marked)																														
	Supraclavicular excavation									■						■	■										■				■
	Supraclavicular (mild/moderate)																				■										
	Supraclavicular (marked)																														
	Upper chest retraction																														
	Lower chest retraction																														
	Tracheal tug	■																											■		
	Abdominal breathing																														
	Thoracoabdominal paradox																									■					
	Suprasternal/tracheosternal									■	■		■		■			■											■		
	Sternocleidomastoid																														
	Scalene																	■													
	Substernal/xiphoid retraction									■						■	■														
	Head bobbing	■								■																					
Dyspnoea	General dyspnoea/breathlessness				■														■		■		■			■					■
	Orthopnoea				■																										
	Speech impairment				■											■	■														■
Nasal flaring	General nasal flaring									■	■	■	■		■							■									
	Mild/infrequent																														

Overall signs observed	Subtype of sign	McCallum *et al.* 2013	Meiias *et al.* 2013	Obata *et al.* 1992	Ochoa Sangrador *et al.* 2012	Pancham *et al.* 2016	Pavón *et al.* 1999	Ralston *et al.* 2010	Reed *et al.* 2012	Rivera-Sepulveda *et al.* 2021	Schuh *et al.* 1990	Walsh *et al.* 2004	Wang *et al.* 1992	McCallum *et al.* 2013	Meiias *et al.* 2013	Ater *et al.* 2012	Bano *et al.* 2018	Chalut *et al.* 2000	Parkin *et al.* 1996	Tal *et al.* 1990	Connett *et al.* 1993	Davis *et al.* 1977	Dev *et al.* i 1997	DiGiulio *et al.* 1993	Ducharme *et al.* 1997	Freelander *et al.* 1984	Giordano *et al.* 2012	Gorelick *et al.* 2004	Hambleton *et al.* 1979	Hurwitz *et al.* 1984	Kamps *et al.* 2014
	Moderate/intermittent																														
	Marked/persistent																														
	Severe/persistent																														
Colour/cyanosis	Colour (pale/mottled)																														
	Cyanosis				■																										
	Circumoral/perioral cyanosis						■																								
	Cyanosis at rest						■																								
	Cyanosis with crying/on exertion						■																								
	Generalised cyanosis						■																								
	Central cyanosis											■																			
	Peripheral cyanosis											■																			
	Mild cyanosis																														
	Moderate cyanosis																														
	Severe cyanosis																														
Behaviour	Mental status/cerebral function				■																									■	
	Awareness/conciousness			■									■																		
	Drowsy/confused												■																		
	Coma/obtunded				■																									■	
	Distress																				■										
	Anxiety																				■										

Overall signs observed	Subtype of sign	McCallum *et al.* 2013	Meilas *et al.* 2013	Obata *et al.* 1992	Ochoa Sangrador *et al.* 2012	Pancham *et al.* 2016	Pavón *et al.* 1999	Ralston *et al.* 2010	Reed *et al.* 2012	Rivera-Sepulveda *et al.* 2021	Schuh *et al.* 1990	Walsh *et al.* 2004	Wang *et al.* 1992	McCallum *et al.* 2013	Meilas *et al.* 2013	Ater *et al.* 2012	Bano *et al.* 2018	Chalut *et al.* 2000	Parkin *et al.* 1996	Tal *et al.* 1990	Connett *et al.* 1993	Davis *et al.* 1977	Dev *et al.* i 1997	DiGiulio *et al.* 1993	Ducharme *et al.* 1997	Freelander *et al.* 1984	Giordano *et al.* 2012	Gorelick *et al.* 2004	Hambleton *et al.* 1979	Hurwitz *et al.* 1984	Kamps *et al.* 2014
	Activity		■		■										■																■
	Agitated/depressed																														
Cardiovascular signs	Heart rate/pulse													■												■					
	Pulsus paradoxus																									■					
Signs of infection	Cough																														
	Cough on stimulus																														
	Spontaneous cough																														
	Occasional cough																														
	Paroxysmal/frequent cough																														
	Mild cough																														
	Moderate cough																														
	Severe cough																														
	Fever																														
	Rhinorrhoea																														
	Mild rhinorrhoea																														
	Moderate to severe rhinorrhoea																														
	Airway secretions																														
	Hoarseness																														
Feeding/dehydration	Feeding - eating and drinking		■						■			■			■																■
	Appetite																														■

Overall signs observed	Subtype of sign	McCallum *et al.* 2013	Meiias *et al.* 2013	Obata *et al.* 1992	Ochoa Sangrador *et al.* 2012	Pancham *et al.* 2016	Pavón *et al.* 1999	Ralston *et al.* 2010	Reed *et al.* 2012	Rivera-Sepulveda *et al.* 2021	Schuh *et al.* 1990	Walsh *et al.* 2004	Wang *et al.* 1992	McCallum *et al.* 2013	Meiias *et al.* 2013	Ater *et al.* 2012	Bano *et al.* 2018	Chalut *et al.* 2000	Parkin *et al.* 1996	Tal *et al.* 1990	Connett *et al.* 1993	Davis *et al.* 1977	Dev *et al.* i 1997	DiGiulio *et al.* 1993	Ducharme *et al.* 1997	Freelander *et al.* 1984	Giordano *et al.* 2012	Gorelick *et al.* 2004	Hambleton *et al.* 1979	Hurwitz *et al.* 1984	Kamps *et al.* 2014
	Dehydration													■																	
Palpation	**Resonance**																														
	Hyperinflation																				■										
	Liver and spleen																														
Demographics	**Age of child**													■																	
	Duration of illness																														
	Weight of child								■																						
Overall severity	**General impression of severity/general condition**														■										■						

Overall signs observed	Subtype of sign	Kelly *et al.* 2000	Kornberg *et al.* 1991	Kudukis *et al.* 1997	Magpuri *et al.* 2018	Moody *et al.* 2020	Needleman *et al.* 1995	Pabon *et al.* 1994	Pendergast *et al.* 1989	Qureshi *et al.* 1998	Rivera *et al.* 2006	Rushton *et al.* 1982	Scarfone *et al.* 1993	Singh *et al.* 1990	Singhi *et al.* 2014	Smith *et al.* 2002	Stevens *et al.* 2003	Uong *et al.* 2018	Vichyanond *et al.* 2013	Bierman *et al.* 1974	Carroll *et al.* 2005	Conway *et al.* 1985	Hussein *et al.* 1986	Kerem *et al.* 1990	Liu *et al.* 2004	McKenzie *et al.* 1979	Williams *et al.* 2011	Wishaupt *et al.* 2017	Wood *et al.* 1972	Yung *et al.* 1996
Respiratory rate	**Respiratory rate/tachypnea**	■				■	■		■	■			■		■	■	■	■	■	■	■		■	■	■		■	■		
Respiratory support	**O2 saturation/hypoxia**	■		■	■	■		■		■	■		■	■	■			■	■		■						■	■		
	Supplemental oxygen	■		■		■		■		■																			■	
	Assisted ventilation																													
Auscultation	**Wheeze/rhonchi**								■			■					■						■					■		
	Wheeze location																													
	Inspiratory wheeze	■			■	■	■			■					■	■		■	■	■	■	■		■	■	■	■			■
	Partial inspiratory wheeze																													
	Full inspiratory wheeze																													
	Inspiratory breath sounds		■										■																■	
	Unequal inspiratory breath sounds			■				■			■																		■	
	Decreased/absent inspiratory breath sounds			■				■			■																		■	
	Expiratory wheeze	■	■	■	■	■	■	■		■	■		■		■			■	■	■	■	■		■	■	■	■			■
	Mild expiratory wheeze																													
	Moderate expiratory wheeze																												■	
	Severe/marked expiratory wheeze																												■	
	Full/pan expiratory wheeze															■														
	End expiratory wheeze	■	■			■	■			■			■		■	■		■		■	■			■	■		■			
	Crackles/rales																													
	Adventitial sounds																													
	Air entry/air exchange/breath sounds	■				■				■								■							■		■			
	Reduced/poor air entry		■		■																	■								

Overall signs observed	Subtype of sign	Kelly *et al.* 2000	Kornberg *et al.* 1991	Kudukis *et al.* 1997	Magpuri *et al.* 2018	Moody *et al.* 2020	Needleman *et al.* 1995	Pabon *et al.* 1994	Pendergast *et al.* 1989	Qureshi *et al.* 1998	Rivera *et al.* 2006	Rushton *et al.* 1982	Scarfone *et al.* 1993	Singh *et al.* 1990	Singhi *et al.* 2014	Smith *et al.* 2002	Stevens *et al.* 2003	Uong *et al.* 2018	Vichyanond *et al.* 2013	Bierman *et al.* 1974	Carroll *et al.* 2005	Conway *et al.* 1985	Hussein *et al.* 1986	Kerem *et al.* 1990	Liu *et al.* 2004	McKenzie *et al.* 1979	Williams *et al.* 2011	Wishaupt *et al.* 2017	Wood *et al.* 1972	Yung *et al.* 1996
	Absent air entry/inaudible/silent chest		■		■						■		■		■					■						■				■
	Aeration (fields)				■																									
	Audible wheeze (without stethoscope)				■								■	■		■				■		■				■				■
I:E ratio	Inspiration to expiration ratio				■								■							■	■									
	Prolongation of expiration (I<E)																■													
Grunting	General grunting																													
	Mild/infrequent																													
	Moderate/intermittent																													
	Marked/persistent																													
	Severe/persistent																													
Apnoea	Apnoea																											■		
Inspection	General accessory muscle use/retractions		■	■			■	■			■	■	■	■			■		■	■	■	■		■		■		■	■	■
	Intercostal	■			■	■	■			■					■			■							■		■			
	Superior intercostal																													
	Inferior intercostal																													
	Intercostal (mild/moderate)																													
	Intercostal (marked)																													
	Subcostal				■										■			■												
	Subcostal (mild/moderate)																													
	Subcostal (marked)																													
	Supraclavicular excavation	■				■			■	■								■							■		■			
	Supraclavicular (mild/moderate)																													

Overall signs observed	Subtype of sign	Kelly *et al.* 2000	Kornberg *et al.* 1991	Kudukis *et al.* 1997	Magpuri *et al.* 2018	Moody *et al.* 2020	Needleman *et al.* 1995	Pabon *et al.* 1994	Pendergast *et al.* 1989	Qureshi *et al.* 1998	Rivera *et al.* 2006	Rushton *et al.* 1982	Scarfone *et al.* 1993	Singh *et al.* 1990	Singhi *et al.* 2014	Smith *et al.* 2002	Stevens *et al.* 2003	Uong *et al.* 2018	Vichyanond *et al.* 2013	Bierman *et al.* 1974	Carroll *et al.* 2005	Conway *et al.* 1985	Hussein *et al.* 1986	Kerem *et al.* 1990	Liu *et al.* 2004	McKenzie *et al.* 1979	Williams *et al.* 2011	Wishaupt *et al.* 2017	Wood *et al.* 1972	Yung *et al.* 1996
	Supraclavicular (marked)																													
	Upper chest retraction																													
	Lower chest retraction																													
	Tracheal tug																													
	Abdominal breathing																													
	Thoracoabdominal paradox						■		■																					
	Suprasternal/tracheosternal				■										■			■												
	Sternocleidomastoid								■						■	■														
	Scalene				■				■																					
	Substernal/xiphoid retraction	■				■				■								■							■		■				
	Head bobbing																													
Dyspnoea	General dyspnoea/breathlessness								■								■		■				■	■	■					
	Orthopnoea																													
	Speech impairment	■								■					■		■										■			
Nasal flaring	General nasal flaring						■																							
	Mild/infrequent																													
	Moderate/intermittent																													
	Marked/persistent																													
	Severe/persistent																													
Colour/cyanosis	Colour (pale/mottled)																												■	
	Cyanosis											■		■									■						■	
	Circumoral/perioral cyanosis																													

Overall signs observed	Subtype of sign	Kelly *et al.* 2000	Kornberg *et al.* 1991	Kudukis *et al.* 1997	Magpuri *et al.* 2018	Moody *et al.* 2020	Needleman *et al.* 1995	Pabon *et al.* 1994	Pendergast *et al.* 1989	Qureshi *et al.* 1998	Rivera *et al.* 2006	Rushton *et al.* 1982	Scarfone *et al.* 1993	Singh *et al.* 1990	Singhi *et al.* 2014	Smith *et al.* 2002	Stevens *et al.* 2003	Uong *et al.* 2018	Vichyanond *et al.* 2013	Bierman *et al.* 1974	Carroll *et al.* 2005	Conway *et al.* 1985	Hussein *et al.* 1986	Kerem *et al.* 1990	Liu *et al.* 2004	McKenzie *et al.* 1979	Williams *et al.* 2011	Wishaupt *et al.* 2017	Wood *et al.* 1972	Yung *et al.* 1996
	Cyanosis at rest																													
	Cyanosis with crying/on exertion																													
	Generalised cyanosis																													
	Central cyanosis																													
	Peripheral cyanosis																													
	Mild cyanosis																													
	Moderate cyanosis																													
	Severe cyanosis																													
Behaviour	Mental status/cerebral function			■				■			■																		■	
	Awareness/conciousness																						■							
	Drowsy/confused																													
	Coma/obtunded			■				■			■																		■	
	Distress																													
	Anxiety																													
	Activity																													
	Agitated/depressed			■				■			■																		■	
Cardiovascular signs	Heart rate/pulse								■												■	■		■						
	Pulsus paradoxus								■															■						
Signs of infection	Cough																											■		
	Cough on stimulus																													
	Spontaneous cough																													
	Occasional cough																													

Overall signs observed	Subtype of sign	Kelly *et al.* 2000	Kornberg *et al.* 1991	Kudukis *et al.* 1997	Magpuri *et al.* 2018	Moody *et al.* 2020	Needleman *et al.* 1995	Pabon *et al.* 1994	Pendergast *et al.* 1989	Qureshi *et al.* 1998	Rivera *et al.* 2006	Rushton *et al.* 1982	Scarfone *et al.* 1993	Singh *et al.* 1990	Singhi *et al.* 2014	Smith *et al.* 2002	Stevens *et al.* 2003	Uong *et al.* 2018	Vichyanond *et al.* 2013	Bierman *et al.* 1974	Carroll *et al.* 2005	Conway *et al.* 1985	Hussein *et al.* 1986	Kerem *et al.* 1990	Liu *et al.* 2004	McKenzie *et al.* 1979	Williams *et al.* 2011	Wishaupt *et al.* 2017	Wood *et al.* 1972	Yung *et al.* 1996
	Paroxysmal/frequent cough																													
	Mild cough																													
	Moderate cough																													
	Severe cough																													
	Fever																											■		
	Rhinorrhoea																											■		
	Mild rhinorrhoea																													
	Moderate to severe rhinorrhoea																													
	Airway secretions																													
	Hoarseness																													
Feeding/dehydration	**Feeding - eating and drinking**																													
	Appetite																													
	Dehydration																													
Palpation	**Resonance**																													
	Hyperinflation																									■				
	Liver and spleen																													
Demographics	**Age of child**																													
	Duration of illness																											■		
	Weight of child																													
Overall severity	**General impression of severity/general condition**						■																							

Appendix 6 – Cochrane risk of bias version 2 assessment of the RCTs eligible for inclusion

Key: green/+ = low risk of bias, yellow/? = some concerns, red/- = high risk of bias.

Author name	Year	1a: Risk of bias arising from the randomization process	1b: Risk of bias arising from the timing of identification or recruitment of participants (cluster-randomized trial only)	2: Risk of bias due to deviations from the intended interventions	3: Risk of bias due to missing outcome data	4: Risk of bias in measurement of the outcome	5: Risk of bias in selection of the reported result	Overall risk of bias
Ater *et al.* [145]	2012	+	N/A	?	+	+	+	?
Bano *et al.* [192]	2018	+	N/A	?	+	?	+	?
Berger *et al.* [195]	1998	+	N/A	-	+	+	+	-
Bogie *et al.* [197]	2007	+	N/A	-	+	+	+	-
Ejaz *et al.* [216]	2015	+	N/A	?	+	?	+	?
Groothuis *et al.* [223]	1990	+	N/A	?	+	+	+	?
Hambleton *et al.* [225]	1979	+	N/A	?	+	+	?	?
Kornberg *et al.* [232]	1991	+	N/A	?	+	?	+	?
Kudukis *et al.* [233]	1997	+	N/A	-	+	+	+	-
Lowell *et al.* [236]	1987	+	N/A	?	+	+	+	?
Moody *et al.* [242]	2020	?	N/A	?	+	+	+	?
Needleman *et al.* [243]	1995	+	N/A	?	+	+	+	?
Scarfone *et al.* [257]	1993	+	N/A	-	?	+	+	-
Tal *et al.* [265]	1990	+	N/A	?	+	+	+	?
Bentur *et al.* [193]	1992	+	N/A	?	+	+	+	?

Author name	Year	1a: Risk of bias arising from the randomization process	1b: Risk of bias arising from the timing of identification or recruitment of participants (cluster-randomized trial only)	2: Risk of bias due to deviations from the intended interventions	3: Risk of bias due to missing outcome data	4: Risk of bias in measurement of the outcome	5: Risk of bias in selection of the reported result	Overall risk of bias
Can *et al.* [199]	1998	?	N/A	?	+	?	+	?
Dabbous *et al.* [208]	1966	?	N/A	?	+	+	+	?
Daugbjerg *et al.* [209]	1993	?	N/A	-	+	+	+	-
Davis *et al.* [210]	1977	?	N/A	?	+	+	+	?
De Boeck *et al.* [211]	1997	?	N/A	-	+	+	+	-
Devi *et al.* [213]	1997	?	N/A	-	+	+	+	-
DiGiulio *et al.* [214]	1993	?	N/A	-	+	+	+	-
Freelander *et al.* [217]	1984	-	N/A	-	+	?	?	-
Groothuis *et al.* [224]	1993	?	N/A	?	+	+	+	?
Pendergast *et al.* [250]	1989	?	N/A	?	?	?	+	?
Singh *et al.* [259]	1993	-	N/A	-	+	?	+	-
Singh *et al.* [260]	1990	-	N/A	?	+	?	+	-
Bohé *et al.* [198]	2004	+	N/A	?	+	?	+	?
Hussein *et al.* [227]	1986	-	N/A	?	+	?	+	-
Alario *et al.* [189]	1992	+	+	+	+	+	+	+
Bajaj *et al.* [190]	2006	?	?	-	?	?	+	-
Jartti *et al.* [228]	2006	+	+	-	-	+	+	-
Qureshi *et al.* [251]	1998	?	+	+	+	+	+	?
Rivera *et al.* [254]	2006	+	+	?	+	+	+	?

Author name	Year	1a: Risk of bias arising from the randomization process	1b: Risk of bias arising from the timing of identification or recruitment of participants (cluster-randomized trial only)	2: Risk of bias due to deviations from the intended interventions	3: Risk of bias due to missing outcome data	4: Risk of bias in measurement of the outcome	5: Risk of bias in selection of the reported result	Overall risk of bias
Schuh *et al.* [258]	1990	+	+	?	+	+	+	?
Singhi *et al.* [261]	2014	+	+	?	+	?	+	?

Appendix 7 – Newcastle Ottawa Scale assessment of each of the cohort and cross-sectional studies eligible for inclusion[183, 185]

Key: green = low risk (7-9 stars), yellow = moderate risk (5-6 stars) and red = high risk (0-4 stars).

Author	Year	Study design	Selection (maximum 4 stars)	Comparability (maximum 2 stars)	Outcome (maximum 3 stars)	Overall number of stars (maximum 9)
Constantopoulos *et al.* [206]	2002	Cohort	4	0	3	7
Chong *et al.* [203]	2017	Cohort	3	2	3	8
Connett *et al.* [205]	1993	Cohort	3	0	2	5
Conway *et al.* [207]	1985	Cohort	3	0	3	6
Deerojanawong *et al.* [212]	1994	Cohort	3	0	2	5
Gajdos *et al.* [218]	2009	Cohort	3	0	3	6
Giordano *et al.* [220]	2012	Cohort	3	0	2	5
Giugno *et al.* [221]	2004	Cohort	3	0	2	5
Hurwitz *et al.* [226]	1984	Cohort	3	0	2	5
Kamps *et al.* [229]	2014	Cohort	3	0	2	5
Kerem *et al.* [231]	1990	Cohort	3	2	2	7
Lai *et al.* [234]	2003	Cohort	3	0	2	5
Levy *et al.* [10]	2004	Cohort	3	1	2	6
Liu *et al.* [235]	2004	Cohort	3	0	2	5
Macias *et al.* [237]	2015	Cohort	3	2	2	7
McCallum *et al.* [239]	2013	Cohort	3	0	2	5
McKenzie *et al.* [240]	1979	Cohort	4	0	3	7
Mejias *et al.* [241]	2013	Cohort	4	2	2	8
Obata *et al.* [244]	1992	Cohort	3	0	2	5
Parkin *et al.* [248]	1996	Cohort	3	0	2	5
Smith *et al.* [262]	2002	Cohort	3	0	2	5
Stevens *et al.* [264]	2003	Cohort	3	0	2	5

Author	Year	Study design	Selection (maximum 4 stars)	Comparability (maximum 2 stars)	Outcome (maximum 3 stars)	Overall number of stars (maximum 9)
Uong *et al.* [266]	2018	Cohort	3	0	2	5
Vichyanond *et al.* [267]	2013	Cohort	3	0	2	5
Wang *et al.* [269]	1992	Cohort	3	0	2	5
Wishaupt *et al.* [271]	2017	Cohort	4	2	2	8
Wood *et al.* [272]	1972	Cohort	3	0	2	5
Yung *et al.* [273]	1996	Cohort	3	0	2	5
Ducharme *et al.* [215]	1997	Cohort	3	2	2	7
Chalut *et al.* [202]	2000	Cohort	3	0	2	5
Gern *et al.* [219]	2002	Cohort	4	0	2	6
Gorelick *et al.* [222]	2004	Cohort	3	1	3	7
Ralston *et al.* [252]	2010	Cohort	4	0	3	7
Williams *et al.* [270]	2017	Cohort	3	0	2	5
Reed *et al.* [253]	2012	Cohort	4	0	2	6
Rushton *et al.* [256]	1982	Cohort	4	0	2	6
Walsh *et al.* [268]	2004	Cohort	3	2	3	7
Bamberger *et al.* [191]	2012	Cohort	4	0	2	6
Carroll *et al.* [201]	2005	Cohort	3	0	2	5
Coarasa *et al.* [204]	2010	Cohort	3	0	2	5
Kelly *et al.* [230]	2000	Cohort	3	0	3	6
Bentur *et al.* [194]	1990	Cohort	3	0	3	6
Sritippayawan *et al.* [263]	2000	Cross-sectional	3	0	2	5
Rivera-Sepulveda *et al.* [255]	2019	Cross-sectional	2	2	3	7
Ochoa Sangrador *et al.* [245]	2012	Cross-sectional	3	2	3	8
Pavón *et al.* [249]	1999	Cross-sectional	2	2	3	7
Pancham *et al.* [247]	2016	Cross-sectional	3	2	3	8

Appendix 8 – Quality assessment using the JBI Critical Appraisal Checklist for Case Series[186]

Author	Pabon *et al.*[246]
Year	1994
Study type	Case series
Was there clear criteria for inclusion in the case series?	No
Was the condition measured in a standard, reliable way for all participants included in the case series?	No
Were valid methods used for identification of the condition for all participants included in the case series?	Yes
Did the case series have consecutive inclusion of participants?	Unclear
Did the case series have complete inclusion of participants?	Unclear
Was there clear reporting of the demographics of the participants of the study?	Yes
Was there clear reporting of clinical information of the participants?	Yes
Were the outcomes or follow up results of cases clearly reported?	Yes
Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	No
Was statistical analysis appropriate?	Yes
Overall appraisal	Include
Comments	The process of case identification could have been better explained, but a severity score was used so this is eligible for inclusion.

Appendix 9 - Quality Assessment using the JBI Critical Appraisal Checklist for Text and Opinion Papers[187].

Author	Bierman *et al.*[196]	Caritg *et al.*[200]	Magpuri *et al.*[238]
Year	1974	1999	2018
Study type	Guidelines	Guidelines	Development of tool
Is the source of the opinion clearly identified?	No	No	Yes
Does the source of opinion have standing in the field of expertise?	Yes	Yes	Yes
Are the interests of the relevant population the central focus of the opinion?	Yes	Yes	Yes
Is the stated position the result of an analytical process, and is there logic in the opinion expressed?	Yes	Yes	Yes
Is there reference to the extant literature?	Yes	Yes	Yes
Is any incongruence with the literature/sources logically defended?	No	No	Yes
Overall appraisal	Include	Include	Include
Comments	This paper could give more evidence to support the guidelines, and refer more to differences to existing literature, with justification. It is eligible for inclusion as it includes a severity score in the relevant age group.	This paper could give more evidence to support the guidelines. It is eligible for inclusion as it includes a severity score in the relevant age group.	This paper appropriately references the relevant literature and uses a systematic process for item selection. This tool should be evaluated using patients to tests its validity.

Appendix 10 – The percentage of severity scores developed in each age group which measure each domain

	Percentage of severity scores developed in each age group that contain each domain (% to 3 significant figures)			
Domains measured	1-5 years and below	6 months-6 years only	1-5 years and above	General paediatric scores
Respiratory rate	79.6	80.0	58.6	63.6
O2 saturation	29.6	60.0	44.8	27.3
Supplemental	9.09	0.00	20.7	9.09
Auscultation	93.2	100	96.6	100
Audible wheeze	45.5	40.00	20.7	36.4
I:E ratio	9.09	40.00	17.2	18.2
Grunting	9.09	0.00	0.00	0.00
Apnoea	4.55	0.00	0.00	9.09
Accessory muscle	93.2	100	100	90.9
Dyspnoea/speech impairment	9.09	60.0	34.5	36.4
Nasal flaring	47.7	0.00	6.90	0.00
Cyanosis/colour	22.7	0.00	6.90	18.2
Mental	22.7	0.00	20.7	18.2
Heart rate	18.2	0.00	6.90	27.3
Pulsus paradoxus	0.00	0.00	6.90	9.09
Cough/hoarseness	6.82	0.00	0.00	9.09
Fever	2.27	0.00	0.00	9.09
Rhinorrhoea/secretions	4.55	0.00	0.00	9.09
Feeding/dehydration	15.9	0.00	3.45	0.00
Resonance/hyperinflation	2.27	0.00	3.45	9.09
Liver and spleen	4.55	0.00	0.00	0.00
Age or weight of child	4.55	0.00	0.00	0.00
Duration of illness	2.27	0.00	0.00	9.09
Overall severity	6.82	0.00	6.90	0.00