The Effect of Respiratory Syncytial Virus and Rhinovirus on Respiratory Morbidity in Children Under Two Years

Thesis submitted in accordance with requirements of the University of Liverpool the degree of Master of Philosophy (MPhil) by

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Abstract

Acute viral respiratory tract infections caused by respiratory syncytial virus (RSV) and rhinovirus (RV) are a leading cause of morbidity and mortality in infants and pre-school children. These viruses commonly cause bronchiolitis in infancy and viral induced wheeze in older pre-school children. For patients admitted to hospital with bronchiolitis caused by these viral infections, instruments to monitor clinical condition are needed to inform management and to determine the efficacy of novel future treatments. The bronchiolitis group at Alder Hey have recently developed and partially validated a clinical severity score for babies with bronchiolitis; the Liverpool Infant Bronchiolitis Severity Score (LIBSS).

It is becoming increasingly evident that early respiratory viral infection which necessitates hospital admission is associated with long term respiratory conditions such as asthma. Several large epidemiological studies in both Europe and USA have demonstrated that RV infection, particularly with Rhinovirus C (RVC) is particularly important.

Aims:

This research outlined in this thesis has two main aims:

1. To complete the validation of LIBSS in children under two years with bronchiolitis or viral induced wheeze, by firstly assessing the instruments responsiveness to change i.e. how LIBS scores change over time in children with bronchiolitis or viral induced wheeze, and secondly comparing LIBSS to the Paediatric Early Warning System (PEWS), a score used in clinical practice. Other secondary aims included collecting/'scavenging' upper respiratory samples for use at a later stage as part of work investigating potential biomarkers of disease severity.
2. As part of a service evaluation, to examine the impact on subsequent respiratory morbidity of RSV or RV infection necessitating hospital attendance in infants < six months of age.

Results:

LIBSS validation: Unfortunately, because of delays in the Ethics/HRA process, we largely 'missed' the 2016/17 bronchiolitis season. As a consequence, we decided to undertake a pilot study to assess ability to recruit, recruitment rates etc. Eighty percent of parents approached agreed to participate in the study. The mean time taken to complete LIBSS was under 5 minutes. Overall, we recruited 15 participants under 2 years of age, the majority

with viral induced wheeze (73%). Most had mild to moderate disease when first assessed. In total 32 LIBSS were completed and four samples were scavenged from clinical laboratories. The average score was 2 (mild disease) and all children had a LIBSS of 0 on discharge, suggesting that LIBSS was sensitive to changes in health status. A strong positive correlation between LIBSS and PEWS scores on admission was found.

RSV & RV service evaluation: we identified 360 infants under six months who were PCR-positive for RSV and 119 PCR-positive for RV. On first presentation to hospital, RSV-positive infants had more severe disease (measured by oxygen requirement, nasogastric feeds/intravenous fluid, need for high dependency) than those who were RV-positive. However, Kaplan-Meier plots and cox-regression analysis revealed that RV-positivity (rather than RSV-positivity) was an independent factor associated with increased risk of re-attendance to A&E with a respiratory illness; 58% of RV-positive children re-attended A&E within 12 months of their first admission compared to 42% of RSV-positive children, with the mean time taken to re-attend A&E being 204 days for those with RV compared to 267 days for those with RSV. Overall, previously RV-positive children were 1.6 times more likely to re-attend hospital than RSV-positive children.

Need for critical care during first hospitalisation was another independent factor associated with increased risk of re-attendance to A&E with a respiratory illness; 68/479 children required critical care during their first attendance with 65% re-attending hospital a mean of 186 days later. Of the 411 infants who did not require critical care, 43% re-attended A&E a mean of 262 days later. Requiring critical care on first hospital admission increased the risk of re-attending A&E for a respiratory reason in the subsequent 12 months by 1.9 times.

Conclusion:

Although we were not able to formally complete validation of LIBSS, we found that parents are willing to participate in a such a validation study and that the instrument is easy to use. Furthermore, LIBSS appeared to correlate well with PEWS scores.

We found that RSV infection in infants six months and under with ARTI is associated with more severe disease than RV infection. With regards subsequent respiratory morbidity over the 12 months following hospital attendance, counter-intuitively, RV rather than RSV infection was associated with greater and quicker re-attendance to hospital. Unsurprisingly, critical care admission associated with the first hospital attendance was also associated with greater and quicker hospital re-attendance.

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Abbreviations

Abbreviation	
95% CI	95 percent confidence interval
Accident and Emergency	A&E
acute lower respiratory tract infection	ALRTI
adjusted odds ratio	aOR
area under receiver operator curve	AUROC
Avon Longitudinal study of Parents and Children	ALSPAC
Bronchoalveolar lavage	BAL
cadherin-related family member 3	CDHR3
Childhood Origins of Asthma stud	COAST
Children's Hospital of Wisconsin Respiratory Score	CHWRS
congenital heart disease	CHD
COnsensus-based Standards for the selection of health Measurement Instrument	COSMIN
continuous positive airway pressure	CPAP
Copenhagen Prospective Studies of Asthma in Childhood 2000	COPSAC2000
deoxyribonucleic acid	DNA
effort of breathing	EOB
Emergency department	ED
Emergency department unit	EDU
gastro-oesophageal reflux disease	GORD
***Haemophilus influenza* type B**	Hib
hazard ratio	HR
health regulartory unit	HRA
health-related patient reported outcomes	HR-PRO
high dependency unit	HDU
human metapneumovirus	hMPV
intensive care unit	ICU
inter-quartile range	IQR
intercellular adhesion molecule 1	ICAM-1
Liverpool Infant Bronchiolitis Severity Score	LIBSS

low density lipoprotein receptor	LDLR
Lower respiratory tract infection	LRTI
matrix protein	M
Modified Wood's Clinical Asthma Score	MWCAS
nasopharyngeal aspirate	NPA
National Institute of Clinical Excellence	NICE
non-bronchoscopic bronchoalveloar lavage	NB BAL
non-structural protein 1	NS1
non-structural protein 2	NS2
nucleoprotein	N
odds ratio	OR
open reading frame	ORF
p value	p
Paediatric Early Warning System	PEWS
paediatric intensive care unit	PICU
Pearson's Correlation Coefficient	PCC
percentage	%
Perth Childhood Acute Asthma Study	PCAAS
phosoprotein	P
Polymerase chain reaction	PCR
Prevention and Incidence of Asthma and Mite Allergy	PIAMA
Quantitative polymerase chain reaction	qPCR
relative risk	RR
Respiratory Assessment Clinical Score	RACS
Respiratory Distress Assessment Index	RDAI
Respiratory Distress Index	RDI
respiratory syncytial virus	RSV
rhinovirus	RV
ribonucleic acid	RNA
RNA dependent RNA polymerase	L
small hydrophobic protein	SH
Tucson Children Respiratory Study	TCRS
Upper respiratory tract infections	URTI
World Health Organisation	WHO

1. Introduction

In 2010, respiratory illness was the fifth leading cause of death in children between 1 – 4 years, with 23% of fatalities in children between 1 – 59 months worldwide being attributable to pneumonia. In the same year, 7.1% of deaths in children under 4 years in the UK were due to lower respiratory tract infections. As expected, child mortality was lower in the United Kingdom due to its economic status. However, in 2014, the Royal College of Children's Health released a report on childhood mortality which stated the rate of infant mortality in the UK was not declining at the same rate as other European countries. Most childhood deaths occur in infancy with the neonatal period being the most vulnerable time when deaths are largely due to birth specific reasons such as congenital abnormalities (1–4).

1.1 Acute Respiratory Infections

Acute respiratory tract infections are a collection of diseases and clinical syndromes caused by infection that affect the respiratory tract. The respiratory system consists of the nostrils, nasal cavity, pharynx, larynx, trachea, bronchi, and lungs. Infections in the respiratory tract are split into upper and lower at the point of the larynx. (5).

1.2 Upper Respiratory Tract Infections

Upper respiratory infections (URIs) include: the common cold, sinusitis, otitis media), acute pharyngitis, tonsillitis, croup, epiglottitis and laryngitis. Most upper respiratory tract infections are self-limiting and can be managed with analgesia. However, in some cases antibiotics may be required and this is due to bacterial infection e.g. a bacterial tonsillitis or epiglottitis. Although the majority of URIs are self-limiting there are some instances where infection can be life threatening like in epiglottitis where the swelling of the epiglottis can cause airway obstruction and is treated as a medical emergency. URIs can lead to complications such hearing loss and mastoiditis in recurrent otitis media. A severe tonsillitis can cause a peri-tonsillar abscess (quinsy) which can also compromise the airway (5–8)

1.3 Lower Respiratory Tract Infections

1.3.1 Whooping cough

Whooping cough is rare in the UK due to childhood vaccinations against the causative agent *Bordetella pertussis*. Symptoms of whooping cough include bouts of coughing ending with a "whooping" sound, vomiting or cyanosis. Symptoms are usually worse at night or after feeding. Symptoms may cause petechial rash on the cheeks, hernias, conjunctival and retinal haemorrhage. Coughing may persist many weeks post infection (9).

1.3.2 Bronchiolitis

1.3.2.1 Clinical Features of Bronchiolitis

Bronchiolitis is a condition most commonly seen in infancy, caused in ~70% cases by respiratory syncytial virus (RSV). Respiratory symptoms associated with bronchiolitis typically follow a predictable course and start with an upper respiratory tract infection during a prodromal period. Lower respiratory tract symptoms then occur on days 1 – 3 which include increased effort of breathing characterised by chest recession (muscles between the ribs are pulled inwards during breathing), nasal flaring and grunting. On auscultation, crackles and/or wheeze may be heard. Symptoms usually worsen between days 3-5, before recovery over a variable period. Some children, particularly those with underlying respiratory conditions or who are very young, follow a more prolonged course.

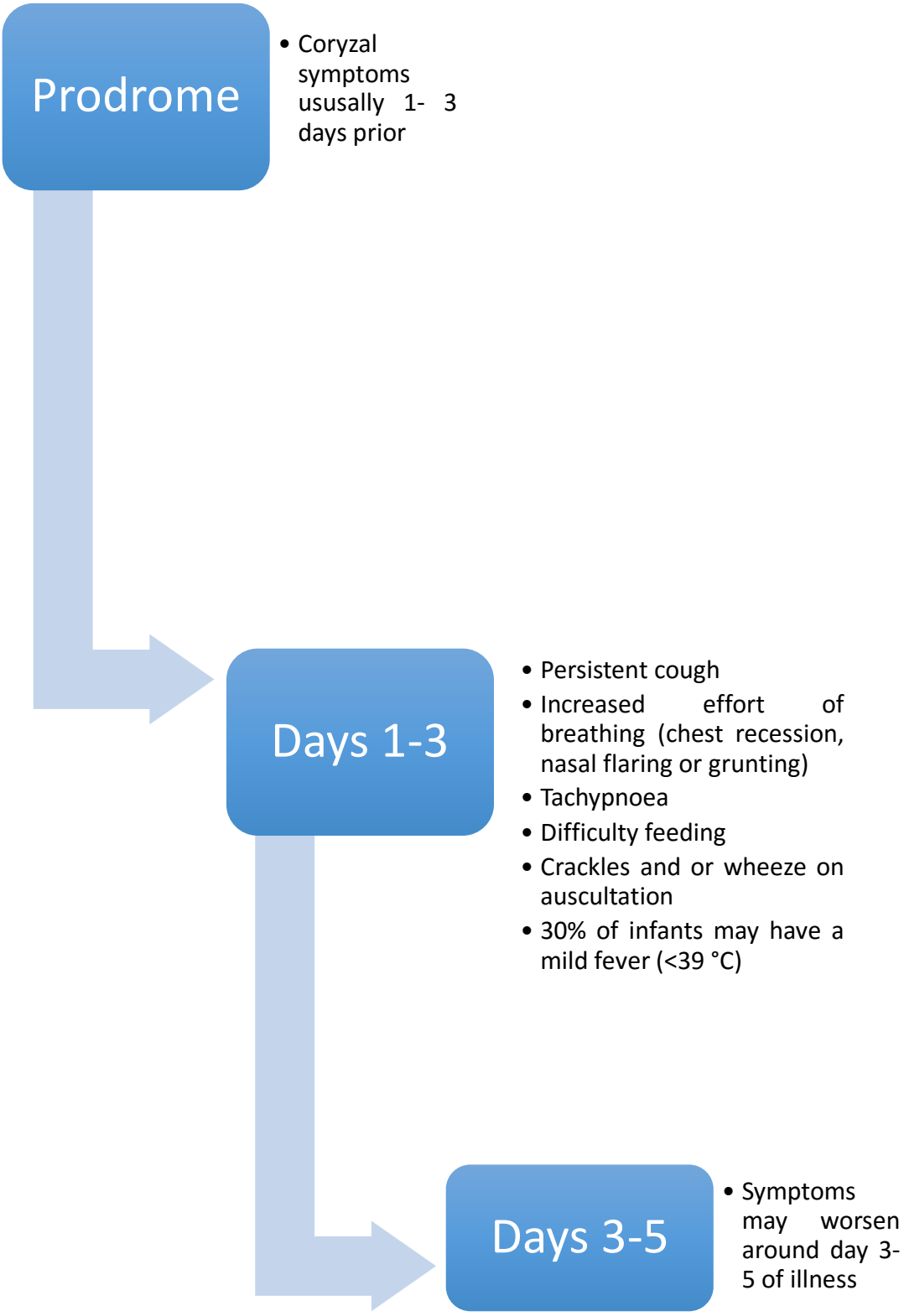

Figure 1 Flow chart of disease progression in bronchiolitis (10)

1.3.2.2 Diagnosis and management of infants with bronchiolitis

The diagnosis of bronchiolitis is based on clinical criteria and supportive investigations. Respiratory samples are often taken in secondary care settings to confirm the presence of common viral causes of bronchiolitis, such as RSV and Rhinovirus (RV). In more severe cases of bronchiolitis where sepsis or another infection is suspected, further samples are taken such as: urine, spinal fluid and blood. These are done to rule out the possibility of a urinary tract infection, meningitis or pneumonia. For those who are severely ill a venous or capillary gas sample will be taken to ensure the patient has not gone into respiratory failure (10–12).

The severity of bronchiolitis varies between children. Table 1 below shows how disease severity can be categorised. Healthy children with mild bronchiolitis can generally be managed at home, although they are sometimes observed in hospital for a few hours. Children with moderate and severe bronchiolitis will be admitted to hospital with more serious cases being referred to the intensive care unit (ICU) (13,14). The treatment of bronchiolitis is mainly supportive as the use of respiratory medications have not shown any benefit. In the last five years, there has been a push to move away from experimental treatment options with no real efficacy through national guidelines. If a child has oxygen saturations less than 92%, oxygen may be given and can be escalated to continuous positive airway pressure (CPAP) if they are nearing respiratory failure. Although NICE recommends an oxygen target of 92% and above, the BIDS study which was carried out in the UK looked at the differences in a target saturation of 90% and above versus 94% and above. Investigators found the time taken for symptoms to resolve was the same in both groups, and feeding returned to normal a median of 2.7 hours earlier in the 90% oxygen target group compared to the 94% group. The change in target saturation did not affect the number of adverse events. This study suggests that oxygen saturation targets for babies with bronchiolitis could be safely lowered, although there are some concerns about how this might affect the developing brain (15).

If breathing difficulties are a result of upper airway secretions or if a child is experiencing apnoea, suctioning may be performed. Nasogastric or orogastric feeding is offered to children who are not taking enough fluids and in cases where this is not tolerated, intravenous fluids may be given. Chest physiotherapy may be beneficial for children with bronchiolitis and neurological disability or tracheomalacia (12).

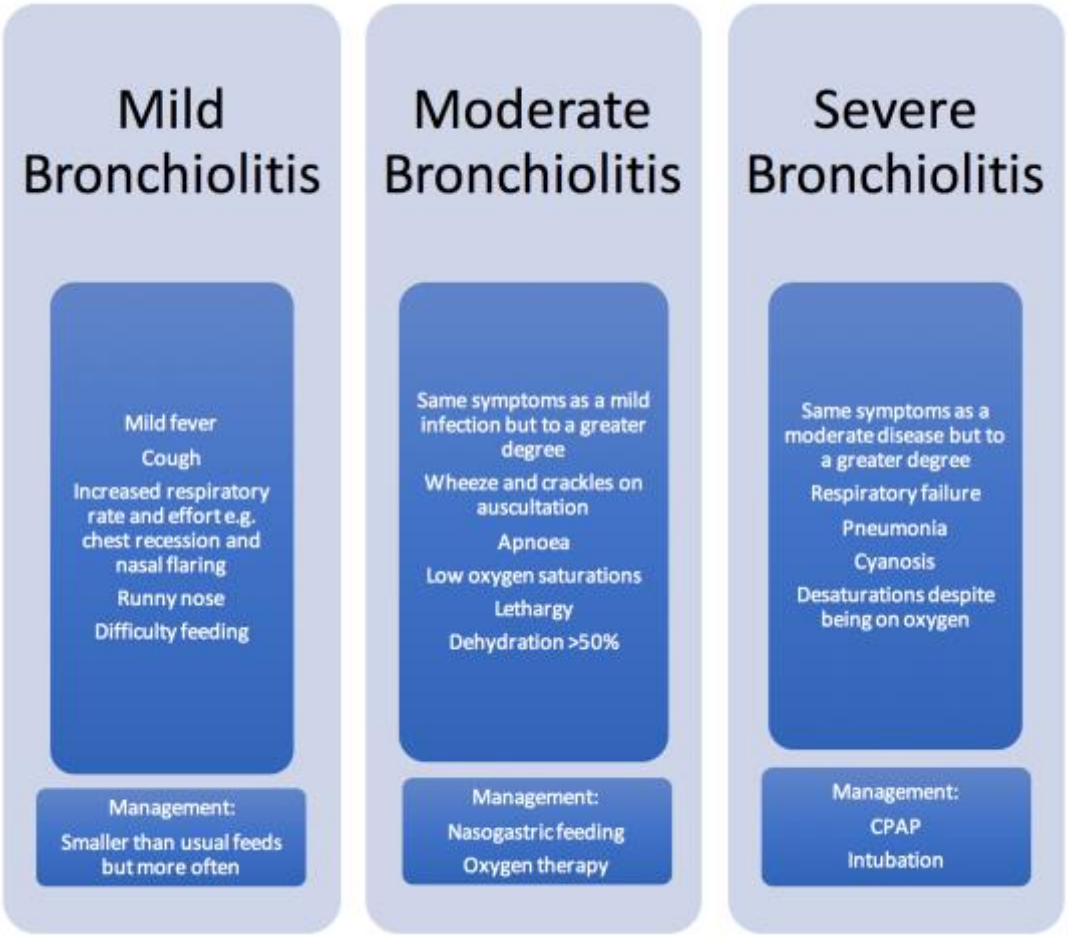

Table 1 *Table of bronchiolitis severity and management* (10,12)

Palivizumab is monoclonal antibody against the cell surface F protein of RSV. The F protein is highly conserved in different RSV species and palivizumab works against RSV A and B. Palivizumab prevents RSV fusing with host cells, and has a half-life of 2-3 weeks so is given to infants every month during bronchiolitis season. Palivizumab is given to children at high risk of severe RSV infection and Public Health England offers guidelines on those who would most benefit. For example, all children under 24 months with severe combined immunodeficiency should be offered palivizumab. In children with haemodynamically unstable heart disease their age at the start of the RSV season and their gestational age at birth are taken into account (16–18).

A formalin inactivated RSV vaccine was trialled in the USA 1965-1966, however the vaccine resulted in more severe symptoms and was associated with two deaths (19). Although much work has subsequently been undertaken trying to develop a vaccine, recently progress in novel antiviral treatments against RSV has been made. For example, Gilead have developed an oral F-protein inhibitor. In human challenge studies, healthy adults were infected with RSV and given the Gilead drug before the onset of symptoms. Investigators found that the drug dramatically reduced mean viral load, and severity of lower respiratory symptoms (20,21). Alios have also developed a RSV polymerase inhibitor(ALS-008176). In a human challenge model, the mean time taken for RSV RNA to be undetected in nasal washes was 1.3 -2.3 days for the intervention groups versus 7.2 days for the placebo group ($p < 0.001$ for all comparisons to placebo). The severity of RSV disease was assessed by means of symptom scores and quantity of nasal mucus production, and was significantly lower in the three dose regiments compared to the placebo group (20,22).

1.3.3 Bronchiolitis Severity Scores

A clinical score enables healthcare workers to confidently identify how severe a patient's illness is and enable the appropriate care to be given. This results in standardised treatment as there is an agreed clinical picture and guidance when to escalate and stop care in timely fashion. Examples of scores used in clinical practice are the Centor Criteria to aid the prescription of antibiotics in acute pharyngitis (23). The CRB-65 predicts how severe community acquired pneumonia is in patients (24). Asthma attacks can also be categorised based on symptoms and this can help guide management (25). A clinical score in bronchiolitis would be ideal as many children deteriorate very quickly. A predictive element like in the CRB-65 system would help eliminate the unpredictability of disease course as well as allow adequate care plans to be put in place. A clinical score would be extremely valuable for research to quantify illness severity and compare the effectiveness of interventions (26).

There are various bronchiolitis severity scores and a systematic review investigated the methodology and validation of each scoring system. A search found 244 articles, however only 14 articles reported on the psychometric properties and the following 11 scoring systems were analysed further (27):

- Respiratory Distress Index (RDI) – abstract only (28)
- Dabbous' Bronchiolitis Score(29)
- Children's Hospital of Wisconsin Respiratory Score (CHWRS) (30)
- Gajdos' Respiratory Score (31)
- Kristjansson Respiratory Score (32)
- Liu Clinical Score (33)
- Respiratory Distress Assessment Index (RDAI) (34)(35)
- Tal Clinical Score (36)(37)
- Modified Tal Clinical Score (37)(38)
- Wang Respiratory Score (32)
- Respiratory Assessment Clinical Score (RACS)(35)

Despite the small number of scoring systems, there was great variability in the content and participant inclusion criteria of these scores. However, all the scoring systems included an assessment of wheeze and use of accessory muscles.(28–38). All the checklist items were guided by opinions of investigators or clinicians and previous scoring systems as opposed to a systematic approach. All the studies assessed for inter-rater reliability, which explores how similar two people's assessment of the same thing is, however not all the studies investigated differences between different types of healthcare professionals. In addition to this the number of individuals used for inter-rate reliability varied amongst the studies (28). This systematic review showed that Respiratory Distress Assessment Index had undergone the most testing for reliability and validity, however there were still flaws with the scoring system (27,35).

Another score of merit is the ReSVinet for healthcare professionals and parents that looks specifically at feeding tolerance, medical intervention, age specific respiratory rate, respiratory difficulty, apnoea and the general condition of a child. The scoring system can be used by all healthcare professionals and in any setting. The parental scale creates a standardised way in which parents can assess their child. This allows an accurate way of informing clinicians about the course of a child's illness at home and a simple way for parents

to identify a deteriorating child. ReSVinet has been assessed for inter-rater reliability, content validity, internal consistency and face validity. Results found that the three clinicians had an inter- observer reliability of >0.7 weighted kappa. Parental scores and investigator 1 were compared and had adequate inter-rater reliability (39,40).

The Liverpool Bronchiolitis Severity Score (LIBSS) was developed with all these issues of validity testing and methodology in mind. Through the LIBSS development the creator Dr. Clare van Miert used the COSMIN checklist to validate the scoring system. The COSMIN checklist aims to provide guidance for those wishing to assess the measurement properties of a scoring system which looks at health related patient reported outcomes (HR-PRO). The checklist gives design and statistical advice so that any conclusions drawn about the properties of a HR-PRO are truly valid (41). Items included in the LIBSS were based on literature reviews of signs, symptoms and risk factors of bronchiolitis. Interviews with parents/carers and workshop discussions of signs and symptoms using the validated Nominal Group Technique and Delphi technique with stakeholders, nurses and other healthcare professionals were also employed. Content validity was carried on 111 infants to assess how difficult healthcare professionals found it to interpret and administer the tool and the time taken. Phase three of development was validity and reliability testing of the score which included construct and content validity testing as well as inter-rater and test-retest reliability. This makes the Liverpool Bronchiolitis Severity Score the first of its kind as has taken into account the COSMIN checklist in its conception and has undergone vigorous testing (27,42).

The Liverpool Infant Bronchiolitis Severity Score measures ten different components for the severity of disease (see Appendix 2 for template). There are two versions of the LIBSS, one for infants under three months and another for infants three months and over, the difference between the two versions are the age specific respiratory and heart rate ranges. The excerpts of the LIBSS in this section are from the under three months version. The first question quantifies observer's/score taker's end of bed assessment of the child in question. Figure 2 is taken directly from the LIBSS and from the end of bed assessment or general impression of the infant in question a maximum score of 8 is given

1. Do you have any concerns relating to the infant's overall condition?			
No concerns (condition is stable or improving)	0		Comments:
Some concerns (may become unstable/requires close observation)	4		
Extremely concerned (unstable requires immediate medical review)	8		

Figure 2 Question 1 of the Liverpool Infant Bronchiolitis Severity Score

The next four questions are respiratory system specific and aim to quantify the level of respiratory distress (Figure 3). Question 2 assesses apnoea, which could be normal in infants or reflect respiratory distress. Question 3 looks at the respiratory effort using accessory muscles and cyanosis. Next is the assessment of oxygen perfusion, which is graded on the ability to maintain a saturation greater than 92% with increasing scores dependent on the administration and quantity of oxygen given. Question 5 looks at respiratory rate and is age dependent.

2. Apnoea				
None		0		Comments:
Occasional self-correcting apnoea / short pauses		2		
Apnoea's increasing frequency & duration		4		
Apnoea's requiring stimulation		6		
Apnoea's requiring bag & mask ventilation		8		
3. Increased work of breathing	**(Absent or Mild =0) Please complete all boxes**			
Moderate/severe recession	0	2		Comments:
Moderate/severe tracheal tug	0	2		
Moderate/severe nasal flare	0	2		
Moderate/severe head bobbing	0	4		
Grunting	0	4		
Central cyanosis (blue lips / tongue)	0	6		
4. % oxygen to maintain saturations ≥92% (or usual saturation level if infant has congenital heart defect)				
21% (room air)		0		Comments:
22 - 40% (0.02 - 6L/min)		2		
41 - 50% (7 - 10L/min)		4		
>50% (>10L/min)		6		
Actual amount of oxygen administered				
Mode of oxygen delivery: Nasal specs (NS); Face Mask (FM); Head box (HB); HiFlow (HF); nCPAP (CP)				
5. Respiratory rate (breaths per minute)				
25 - 59		0		Comments:
60 - 70		2		
<25 or >70		4		

Figure 3: Questions 2 - 5 of the Liverpool Infant Bronchiolitis Severity Score

Question 7 looks at the effect illness has on child's neurological system or energy levels. Question 8 looks feeding habits during illness which is important as many children are admitted for supportive care based on fluid intake. Urine output is also assessed as it can reflect inadequate fluid intake or shock leading to acute kidney injury. Finally, capillary refill is assessed centrally as a rudimentary indicator of perfusion.

The Liverpool Infant Bronchiolitis Severity Score includes all the items in the Paediatric Early Warning System (PEWS) (Figure 4) except for temperature. The PEWS is an observational tool used by nurses to highlight deterioration during hospitalisation. The PEWS gives observations a score and the total prompts nursing staff to request a medical review. PEWS also allows clinicians to see the trend in scores over time which create an overview of a patient's health since they were seen last. PEWS and the adult version NEWS, helps identify patients that are going into shock so that appropriate investigations and management can be given promptly. Developing the LIBSS further to have actions dependent on the score would also be useful.

revised based on pilot study 10/2012

Respiratory rate	**99th centile**	**90th centile**	**Acceptable**	**10th centile**	**1st centile**
Score	2	1	0	1	2
Age 0-1yr	≥ 70	60 – 69	26-59	20-25	< 20
Age 1-2yr	≥ 55	50-54	21-49	15-20	<15
Age 2-7 yr	≥ 40	35-39	21 - 34	15–20	< 15
Age 7-13yr	≥35	≥30	16-29	≤15	≤10
All > 13yr	≥35	≥30	11-29	≤10	≤5

Effort of breathing	Marked subcostal recession, tracheal tug, grunting, nasal flaring, stridor, head bobbing		
Score	0= acceptable	1= any one of above	2= any two + of above

SpO2	≥ 93	88-92	< 88
Score	0	1	2

O2 required	0 = No	1 = Yes	
O2 delivery	≤ 28%	29-50%	> 50%
	0-3 L/min via FM	4-10 L/min via FM	> 10 L/min via FM
Score	0-1L/min via NC	1-2L/min via NC	change delivery

Pulse rate	**99th centile**	**90th centile**	**Acceptable**	**10th centile**	**1st centile**
Score	2	1	0	1	2
Age 0-1yr	≥ 170	160 - 169	101-159	91- 100	≤ 90
Age 1-2yr	≥ 165	155 - 164	101-154	81- 100	≤80
Age 2-7 yr	≥ 140	135 - 139	91-134	81–90	≤80
Age 7-13yr	> 130	125-130	71 -124	61–70	≤ 60
All > 13yr	≥ 130	120–139	61-119	51–60	≤ 50

Blood pressure	Score 10 if systolic BP is on or below 5th centile for age
Age 0-1yr	≤ Systolic 55
Age 1-2yr	≤ Systolic 65
Age 2-7 yr	≤ Systolic 70
Age 7-13yr	≤ Systolic 75
All > 13yr	≤ Systolic 80

Capillary refill T	1-2 seconds	3-4 seconds	> 4 seconds
Score	0	1	2

AVPU	Alert	Verbal	Pain	Unresponsive
Score	0	1	3	10

Nurse concern	Score 1: if nurse concerned child is deteriorating
Parent concern	Score 1: if parent is concerned that child is deteriorating

Action to be taken for PEW scores
Score 0 - 2 . Accept as variant of normal. Observations minimum 6 hrly
Score 3- 5. Review by nurse in charge (NIC) of ward. Take a complete set of obs within 1/2 hour If repeat score is 3 -5 , increase the frequency of obs. If the NIC is concerned about the patient get a medical review within one hour. SHO/F2 discuss case with Reg. Agree plan with NIC
Score 6 - 9. Review by nurse in charge (NIC) of ward. Take a complete set of obs within 10 mins If repeat score is 6 -9 , increase the frequency of obs. Start continuous monitoring Get urgent medical review within 30 mins. Agree plan with NIC & Reg
Score 10+. Review by nurse in charge (NIC) of ward. Take a complete set of obs immediately. Continuous monitoring. Urgent review by registrar or above within 10 minutes If cannot contact own team - call 2222
Use SBAR to communicate critical information in a succinct and structured format
Paediatric Early Warning systems do not replace your clinical judgement. It is an adjunct. If a patients is deteriorating rapidly - call 2222

Figure 4 Paediatric Early Warning System

1.3.4 Pneumonia

Pneumonia is a lower respiratory tract infection typically caused by bacteria but can be caused by viruses and fungi. The World Health Organisation (WHO) reported in 2015 that 16% of all deaths in children under five worldwide were caused by pneumonia (43). NICE Clinical Knowledge Summaries recommends pneumonia should be considered in children with fever, age specific tachypnoea, crackles on auscultation, nasal flaring chest recession, cyanosis or oxygen saturations <95% in room air (44). The management of pneumonia is based on the severity of illness. If a child is well enough to be cared for at home, parents should be given information about managing fever and preventing dehydration. 'Safety-netting' ensures that parents can recognise signs of deterioration and the appropriate place to seek help. Children under two years with mild lower respiratory tract symptoms do not need a course of antibiotics. However, if there are clear signs of pneumonia, antibiotics should be given as there is no way to distinguish between viral and bacterial pneumonia. Amoxicillin is the empirical choice of antibiotic in children as it is effective against the most common bacterial pathogens. If a hospitalised child requires intensive care, attempts to identify the causative organism should be made. This is done by:

- Nasal pharyngeal aspirate (NPA)
- nasal swabs
- blood cultures
- serology for respiratory viruses and *Mycoplasma* and *Chlamydia*

If a child does not improve within 48 hours in hospital or in the community re-examining the child should be considered. In a hospital setting, children with severe pneumonia, empyema or lung abscesses should be monitored after discharge until their x-rays return to near normal (45,46).

	Mild to Moderate Pneumonia	**Severe Pneumonia**	**Other symptoms**
Infants	Fever Respiratory rate <50 breaths per minute Mild chest recession Still managing full feeds	Fever Moderate/ severe chest recession Nasal flaring Grunting Cyanosis Intermittent apnoea Reduced feeding Capillary refill time ≥ 2 seconds Tachycardia appropriate for patient's age	
Older Children	Fever Respiratory rate <50 breaths per minute Mild breathlessness	Fever Respiratory rate >50 breaths per minute Severe difficulty breathing Nasal flaring Cyanosis Grunting Signs of dehydration Intermittent apnoea Reduced feeding Capillary refill time ≥ 2 seconds	Pleuritic pain Abdominal pain

		Tachycardia appropriate for patient's age	

Table 2 Signs and symptoms of pneumonia based on age(43–45)

1.3.5 Viral Induced Wheeze

Viral induced wheeze occurs in children between 6 months to five years. Diagnosis is usually based on history of viral upper respiratory tract symptoms, the age of the child and the presence of recurrent episodes of wheeze (12). Signs and symptoms can be very similar to that of bronchiolitis and in truth, the differentiation between the two conditions is likely to be artificial given that both tend to be caused by the same pathogens.

In 2008, the European Respiratory Society published evidence based definitions for different wheezing phenotypes. Viral induced wheeze or episodic wheeze was defined as:

"Episodic (viral) wheeze is defined as wheeze in discrete episodes, with the child being well between episodes (47)*."*

Wheezing itself is not disease or syndrome but a symptom. It is defined as a high-pitched whistling sound associated with breathing indicative of airway narrowing. Viral induced wheeze is common in preschool children and is associated with an acute upper respiratory infection. Episodic wheezing usually disappears by the age of 6. Multiple-trigger wheeze or persistent wheeze is defined as children who wheeze in response to triggers. Triggers include: tobacco smoke, allergen exposure, mist, laughter and exercise. However emerging evidence shows these two definitions do not fully encapsulate the course of preschool wheezing. Viruses such as RSV and rhinovirus have been linked to increase wheezing over time (47–50).

There are no clear treatment options for viral induced wheeze. It is recommended that children with mild intermittent wheeze with respiratory tract infections should not have any treatment and be reviewed later. Parents are advised to check on their child regularly for signs of deterioration. Children that are moderately sick should be given a bronchodilator via spacer and reviewed 15-30 minutes later. Responsive children can be managed at home, and those who are severely ill or do not respond to bronchodilators must be admitted for supportive care such as oxygen. Children over two years may benefit from a bronchodilator such as a short acting beta-agonist Children who have previously responded to bronchodilators may be prescribed a bronchodilator for future episodes only (44).

1.4 Acute Respiratory Infection Pathogens

Streptococcus pneumoniae (*S. pneumoniae*) is the leading bacterial cause of childhood pneumonia. There are various strains of *S. pneumoniae* and in the UK childhood vaccinations are offered against 13 stains (45). This has led to a reduction in the laboratory confirmed incidences of these bacterium however there is an increase in those not covered (51,52). *Group A streptococcus* is associated with an increased risk of attending ICU and empyema development. *Staphylococcus aureus* and influenza coinfection is associated with an increased disease severity. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are atypical causes of a lower respiratory tract infection. Viruses are a common cause of lower respiratory tract infections in the young and oftentimes a coinfection between a bacterial and viral organisms is found (45). RSV is the leading viral cause of lower respiratory tract infections in children under one years, rhinovirus is the leading cause in older infants. Other viruses of note are, parainfluenzas 1 -4, influenza A and B, human metapneumovirus and human bocavirus (10,46,53).

1.4.1.1 Respiratory syncytial virus (RSV)

RSV belongs to the *Paramyoxviridae* family alongside other viruses such as measles and mumps. The *Paramyoxviridae* family are all negative sense RNA viruses, RSV and human metapneumovirus are then grouped together to make the genus (subfamily) *pneumovirus*. RSV is then split into two species - A and B but there is still great variability between individual viruses. The RSV genome is approximately 15,200 nucleotides in length (54). The genome codes for 10 genes and the sequence: NS1-NS2-N-P-M-SH-G-F-M2-L. The negative sense RNA is surrounded by nucleoprotein (N), phosphoprotein (P) and RNA dependent RNA polymerase (L), these three proteins and RSV RNA form a ribonucleoprotein (RNP) complex. The RNP structure protects the inner RNA and all are necessary for successful replication. RNA replication involves transcribing a complementary single stranded positive-sense copy, this is known as an antigenome (55–57).

RSV RNA is enclosed in a lipid bilayer with small hydrophobic (SH) proteins, glycoprotein G and F on its outer surface and matrix (M) protein on the inner layer which is involved in RSV replication, assembly and packaging (58,59). Glycoprotein G is responsible for attachment to host cells, fusion (F) glycoprotein is responsible for fusion with host cells and forming a

syncytia with neighbouring cells (60). With F glycoprotein alone host cells can be infected but G glycoprotein increases efficiency of infectivity (61). The small hydrophobic glycoprotein is somehow involved in inhibiting apoptosis in infected host cells (62). Non-structural protein 1 and 2 (NS-1 & NS-2) are not essential for replication and are produced by host cells. One way in which the NS proteins promote virulence is by delaying apoptosis and consequently increase viral load in host cells (63). The section of RNA contains the M2 code has two overlapping open reading frames (ORF-1 and ORF-2) which translate into M2-1 and M2-2. M2-1 is a transcription elongation factor and M2-2 is helps switch from RNA transcription to replication (64–66).

1.4.1.2 Epidemiology of RSV

Respiratory syncytial virus (RSV) primarily affects the young, immunocompromised and the elderly. Looking at the data provided by Public Health England most RSV laboratory cases are from children under five years with most cases being infants. The second peak is seen in adults over 65 years (10,67). Annually infection peaks seem to correlate with wet or cooler months. In the UK RSV peaks from October to March (55,68,69).

The total burden of RSV is difficult to quantify for a number of reasons. Firstly, RSV infection may be mild and not require medical care and subsequent viral testing. Secondly, different hospitals have different policies or clinicians may admit on clinical signs and symptoms and not carry out viral testing. The estimation of the RSV burden comes from data collected by anonymous or linked hospital and primary care statistics, mortality data and epidemiology research. A systematic review of literature estimated that there were 33.8 million episodes of respiratory syncytial virus associated LRTI globally in children under five years and it makes up 22% of all acute lower respiratory illness in children. The incidence was double in developing countries versus developed countries such as the UK (70).

Looking specifically at the UK several studies using various methods have assessed the RSV burden (Table 3). Between 2007-2012, 33,561/121,968 (27.5%) of estimated respiratory hospitalisations in children under five years were associated with RSV in children four years and under. Infants under 6 months were the age group most likely to be admitted to hospital with a RSV infection (71).

Study	Method	Data	RSV burden
Hardelid *et al.* 2013 (72)	Multivariable regression model	Mortality data from ONS Health Protection Agency laboratory reports on influenza A and B and RSV in England and Wales	597 season deaths attributed to RSV in children under 15 years from the years 1999 to 2010 1.8% of total deaths
Deshpande *et a.l* 2003 (73)	Linked data so reported descriptively	Shropshire Health Authority database: microbiology and hospital episodes Child Health System database	2.4 per 100 hospitalisations for infants residing in Shropshire from 1996 – 1999 were RSV related, 16.3 per 1000 in children under 2 years
Reeves *et al.* 2017 (71)	Multiple linear regression models	HES data Laboratory surveillance	33,561/121,968 of estimated respiratory hospitalisations in children under five years were associated with RSV in children four years and under
Taylor *et al. 2016* (74)	Multiple linear regression model	Weekly Public Health England viral surveillance data Clinical Practice Research Datalink HES Mortality data	14,441/10,000, (14.2%) of all GP visits in infants under six months 4,184 per 100,000 (42.2%) of hospitalisations in infants under six months From 1997 - 2009 12% of deaths and 17.6% years 1996 - 2009

Table 3 Summary of studies estimating RSV burden (71–74)

1.4.1.3 Rhinovirus

Human rhinoviruses (RV) are the leading viral cause of acute respiratory infections. There are three species; RV A, B and C. RV-C was only discovered in 2009 as RV-C strains do not grow in standard viral culture media. RVs cause an array of diseases such as the common cold, sinusitis, bronchiolitis and pneumonia (75,76).

RV belongs to the *enterovirus* genus and *picornaviridae* family and is a positive sense single stranded RNA virus. The RV genome is 7,200 base pairs in size and codes for a single gene that translates into 11 proteins. Four of the eleven proteins (VP1-4) make up the capsid that envelopes the RNA (77,78). The remaining seven proteins are involved in virus replication and assembly. There are over 100 serotypes of RV, with serotypes being attributed to differences in capsid proteins VP1, VP2 and VP3; the remaining capsid protein VP4 secures the RNA core to the capsid. Sixty copies of each virus join to form an icosahedral shape with each viral VP1 protein containing a canyon which acts as an attachment site for the virus to attach to cells. Rhinovirus is transmitted between individuals through direct contact, i.e. fomites and small or large aerosol particles. Experiments have shown that RV can last between hours and days in an indoor environment and for up to two hours on the skin (75,76). Most RV serotypes use the surface receptor intercellular adhesion molecule 1 (ICAM-1) to attach to host cells and may be aided by heparin sulphate. Approximately 10% of serotypes use a low-density lipoprotein receptor (LDLR) to enter host cells. Recent studies have shown that cadherin-related family member 3 (CDHR3), found in lower respiratory tract cell surfaces is most likely used by RV-C as a receptor to enter host cells (79). A mutation in in CDHR3 has been linked to asthma exacerbations in children. This may explain why a high proportion of asthma exacerbations are caused by RV-C (79–81).

There may be differences in how species interact with host cells due to the differences in genetic material and mode of entry. Some studies have found that the different species cause different severities of disease and clinical presentation. For example, a Moroccan study found that a RV-A infection in children under 5 years was associated with pneumonia, whereas a RV-C infection was associated with wheezing and a higher incidence of oxygen and bronchodilator use (82). Supporting this finding further is the Perth Childhood Acute Asthma Study (PCAAS) where RV-C was the most prevalent type of rhinovirus found in children presenting to A&E with asthma exacerbations. In addition to this, RV-C was associated with more severe disease than that caused by RV A, B or other respiratory viruses (83). Similar findings were also found in a UK retrospective study. RV-C mono-infection was linked to a higher disease severity in children under three years compared to the two other

species (84). Cox *et al.* investigated respiratory wheezing morbidity in children under five years. Investigators found that those who were positive for RV-C at the time of the study were more likely to be admitted before and 12 months after the study commencement with respiratory illness. The relative risk was increased further if a child was atopic (85). A study in non-hospitalised participants also found that RV-C and A were associated with a greater disease severity and that RV-C mainly affected children (86).

1.4.1.4 Epidemiology of RV

Rhinovirus is in circulation all year round but peaks during the autumn with a smaller peak observed in spring. Rhinovirus is the commonest cause of viral respiratory tract infection except in the winter months when influenza and RSV supersede it (76).

1.4.2 Viral Identification

In the UK, polymerase chain reaction (PCR) is the most common way of identifying multiple respiratory viruses in samples. Traditional methods of viral detection include culture, antigen detection and serology. PCR is used clinically over more traditional methods as it is highly sensitive and results can be reported the same day unlike cultures. Rapid antigen testing is used in many hospitals for testing for individual viruses such as influenza and RSV.

PCR involves isolating viral DNA from respiratory samples and then mixing isolated DNA material with specific viral primers. Multiplex PCR testing allows investigators to test multiple viruses in one reaction test tube. However, some sensitivity is lost when multiple primers are used. Quantitative PCR (qPCR) enables users to quantify how much DNA/RNA for each specific virus is in a sample, and multiplex PCR machines can be quantitative or qualitative. This allows viral load to be analysed which has the potential to help differentiate between previous and current respiratory infections (87).

1.4.3 Respiratory Samples

1.4.3.1 Nasal Pharyngeal Aspirates

Nasal pharyngeal aspirates (NPA) are commonly used to detect respiratory viruses in children. An NPA is collected by using a catheter attached to a collection pot with a suction outlet. The catheter, once measured for size, is inserted into a patient's nostril, then suction is applied and respiratory secretions are collected in the attached collection pot (88,89). Pathogens found in the upper respiratory tract may not reflect pathogens causing lower respiratory tract disease. However, in viral lower respiratory tract infections, results found in the upper airways may reflect the lower respiratory tract. Pathogens can also be present in asymptomatic patients so NPA results may not always reflect illness (87).

1.4.3.2 Nasal Swabs

Nasal swabs have been improved by flocking; new flocked swabs are more comfortable when used and collect more epithelial cells. When collecting a swab, a patient should have their head angled back slightly, the swab is then inserted into the nostril and sample is collected from the posterior nasopharynx by rotating swab, this should be done in both nostrils (88,89).

1.4.3.3 Sputum Samples

Sputum sampling is a non-invasive method of collecting respiratory secretions. And is commonly used to help identify bacterial pathogens: *S. pneumoniae, H. influenza and M. catarrhalis.* To ensure that sputum samples truly reflect the lower respiratory tract, a cytological cut-off point has been created that compares polymorphonuclear cells and squamous epithelial cells. In children, it is difficult to collect sputum samples that are purulent enough for appropriate analysis (88).

1.4.3.4 Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) is an invasive technique used to collect samples from the lower respiratory tract. This is an improvement on sputum sampling as it avoids the potential for sample contamination with upper respiratory tract flora. BAL is generally carried out during bronchoscopy, with saline being flushed down the bronchoscope into the airways and then sucked out into a sample pot. Samples can also be collected 'blindly' when a child is intubated on intensive care using a technique known as non-bronchoscopic bronchoalveloar lavage (NB BAL) (88,90).

1.4.4 Risk Factors for Lower Respiratory Tract Infections

1.4.4.1 Prematurity

Prematurity can be defined as infants born before 37 weeks' gestation and has been highlighted as a risk factor for acute lower respiratory tract infections. Severely premature infants (babies born at less than 33 weeks gestation) are at even greater risk (53,91,92). A meta-analysis found that any prematurity resulted in an odds ratio (OR) of 1.96 (95% CI 1.44 – 2.67) for RSV infections in hospitalised children but that prematurity < 33 weeks gestation increased the OR to 2.74 (95% CI 1.59 – 4.71) (93).

1.4.4.2 Pre-existing Medical Conditions

Certain underlying medical conditions are associated with more severe lower respiratory tract infections (LRTIs). A cohort study over eight RSV seasons found that 46% of patients admitted to PICU with bronchiolitis/LRTI caused by RSV had an underlying medical condition such as: chromosomal abnormalities, cardiac lesions, neuromuscular conditions, chronic lung disease (born prematurely or with bronchopulmonary dysplasia, or cystic fibrosis), large airway abnormalities, or immunodeficiency. Overall there were 35 deaths in this cohort and all had a pre-existing medical condition (OR 2.36; 95% CI 2.02 to 2.76). Having a cardiac anomaly was associated with the greatest chance of death (OR 2.98; 95% CI 2.16 to 4.12) (94). Congenital heart disease (CHD) and particularly haemodynamically unstable CHD, increases the risk of severe LRTI as the cardiorespiratory system is already strained, whereas those with cystic fibrosis have difficulty clearing airway secretions from their lungs and are therefore at increased risk of respiratory infections such as pneumonia (53,91). Neuromuscular disorders such as cerebral palsy and chromosomal abnormalities also increase the risk of LRTI (95,96).

1.4.4.3 Male Sex

There are mixed reports about whether the male sex is a risk factor for lower respiratory tract infections or not. El Saleeby *et al.*, found that the male sex was a risk factor for a longer length of hospital stay in children with RSV. However, recent NICE guidance reports that a higher proportion of male children make up those hospitalised for bronchiolitis and community acquired pneumonia, but severity of disease in boys and girls admitted to hospital is similar (53,91,97).

1.4.4.4 Lack of Breastfeeding

Breastfeeding up until the age of six months is recommended by the World Health Organisation. Breast milk contains antimicrobial substances that may help the immature

immune system fight off infections. A cohort study found that breastfeeding for 6 months or more was protective against acute respiratory infections (ARI) (OR 0.71; 95% CI: 0.51–0.98). The protective effect was stronger in studies with shorter follow up periods suggesting that the benefits of breastfeeding are not lifelong (OR at six months 0.50 [95% CI: 0.32–0.79]; OR at 7 – 12 months: 0.46 [95% CI: 0.31–0.69]) (98). Other studies have also found breastfeeding to be protective (99,100).

1.4.4.5 Overcrowding, Siblings and Day Care Attendance

Attending nursery increases the risk of a child encountering other viruses. Babies living with siblings of school age are more susceptible to ARI. One systematic review reported a moderate risk for day care attendance and a severe acute LRTI (OR 1.61; 95% CI 0.98 – 2.64) and the presence of siblings (OR 1.60; 95% CI 1.32 – 1.95) (92). Another systematic review reported much higher odds of RSV LRTI infection in children attending day care (OR 3.7; 95% CI 0.7 to 20.2) (93). A potential reason for the differences between the results of these systematic reviews could be the limited number of studies in industrialised countries in the latter review, or the different outcome measures.

1.4.5 Asthma

Asthma is the commonest chronic disease of childhood, affecting 1 in 11 children in the UK (101). The severity of asthma varies. Some people may only have symptoms with an acute viral infection and will only take bronchodilators to relieve symptoms at that point, whereas others may need daily inhaled corticosteroids to maintain health.

A diagnosis of asthma is based on an individual's history. The clinical features of asthma are: wheeze, cough, difficulty breathing and chest tightness. These symptoms can happen in other diseases however there are set characteristics which make them more likely to be asthma. Asthma symptoms are usually: recurrent, frequent, happen outside colds, worse at night and early morning, and are made worse by triggers such as damp air and pet exposure. A personal or family history of atopy also increases the likelihood of asthma. (102).

1.4.6 Recurrent Wheeze and Asthma Development

A number of prospective cohort studies such as the Tucson Children Respiratory Study (TCRS), the Childhood Origins of Asthma study (COAST) and the Copenhagen Prospective Studies of Asthma in Childhood 2000 high-risk birth cohort (COPSAC2000), have investigated the development of asthma from birth in high risk patients (children with a positive family history for atopy or asthma) (103–105). These studies have helped provide information on the clinical outcomes associated with recurrent preschool wheeze.

The Tucson Children Study recruited infants from parents involved in a study, and they obtained follow up data for 826 children. Parents were advised to seek medical help if their child had symptoms such as a wet cough or wheezing from the chest. Lung function and serum IgE was taken in the first year of life as well as a questionnaire, which was then sent out families at regular intervals. In depth evaluations were carried out at 6, 11, 16, 22, and 26 years, at the first evaluation, investigators found 60% of those who had wheezing episodes in the first three years of life did not wheeze at 6 years. In a minority of children, wheezing in infancy was related to a predisposition to asthma, but these children also had elevated IgE serum levels (106). From the Tucson cohort study, the following concepts have been developed regarding childhood wheezing:

- Transient wheeze: wheezing that resolves by three years and is associated with reduced lung function at birth and is usually associated with a viral infection.

- Atopic wheeze/asthma: wheezing develops later in life than transient wheeze and is associated with atopy and bronchial responsiveness
- Non-atopic wheeze: not as persistent or severe as atopic wheeze

As more cohort studies looking at respiratory health have been published, the concepts regarding the stratification and outcomes of pre-school wheeze have changed. The Avon Longitudinal study of Parents and Children (ALSPAC), collected data from symptom questionnaires on its birth cohort at seven time points to identify wheezing phenotypes and the association with asthma, atopy, airway responsiveness at 7 – 9 years. A latent class method was used to identify wheezing phenotypes. In total 6265 children completed all questionnaires. From this study seven categories for wheezing were created:

- Never/ infrequent wheeze: 59.3% of children
- Transient early wheeze: occurred in 16.3% of children, wheezing from 6 months to 18 months
- Prolonged early wheeze: 8.9% of participants, wheezing from 6 months to 30 months
- Intermediate onset wheeze: 2.7% of participants wheezing peaks from 18 months to 54 months
- Late onset wheeze: 6% of the study population wheezing from 42 months to 69 months
- Persistent wheeze: 6.9% of participants, wheezing from 6 months – 69 months of age

When comparing the never/infrequent wheezing to the other wheezing phenotypes, all categories were associated with physician diagnosed asthma at 91 months of age. The two highest odds ratios reported were persistent wheeze (OR 308 (95% CI 186 – 510)) and intermediate onset of wheezing OR 326 (95% CI 138- 770). To further support this finding airway responsiveness was highest in these two groups. Atopy was associated with intermediate, late onset and persistent wheeze. Intermediate wheezing was strongly associated with airway responsiveness and atopy, investigators concluded that this is the window where environmental factors such as allergens and viral infections and genetic variants in the immune response increase the risk of asthma (107).

A Dutch cohort study used this approach and found similar results. The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort was smaller and came up with a 5-class model. The prolonged early transient wheeze group was lost in this cohort. However

very similar results were found for the newly identified intermediate onset of wheeze and further strengthens the findings (108). The Southampton Women's Survey went on to validate the wheezing phenotypes in their cohort and provide more information about the timing of sensitization, and lung function. Atopy was present by 1 year for intermediate wheezers and was present by three years in late onset or persistent wheezing. Using nitric oxide as an indirect estimate of eosinophilic airway inflammation, investigators found an significant association with nitric oxide and wheezing at six years (109).

The Manchester Asthma and Allergy Study added further knowledge by looking at wheeze alongside other respiratory symptoms as well as allergy sensitization as a continuous variable rather than its mere presence. Results from this investigation found at 3 years, wheeze and coughs were significantly associated with baseline lung function and maternal asthma and child's atopy. At 5 years, wheeze and cough were associated with baseline lung function (p= 0.001), the interaction between maternal asthma and sum of specific IgE (p = 0.003), and the interaction between maternal asthma and child's airway reactivity (p = 0.04) were significantly associated with the components.

1.4.7 Asthma, Recurrent Wheeze and Viral Infections

From the previous section, we can see the different wheezing phenotypes carry varying risks of asthma development. There are various factors that may affect the type of wheeze that is developed. These factors are: age at infection, severity, virus type, and allergic predisposition or sensitisation.

RSV and RV have been shown to increase the odds of recurrent wheeze/asthma development. Blanken *et al*. showed that in healthy premature infants palivizumab versus a placebo reduced the number of RSV infections and the number sick days with respiratory disease for up to one year (110). Other studies have shown that this association continues throughout the preschool years but there are mixed results as children progress to adulthood (111). Sigurs *et al.* compared 46 infants with severe RSV bronchiolitis at less than one year to a control group to determine the risk of asthma and recurrent wheeze over time. The prevalence of asthma increased in both groups, conversely the relative risk of asthma decreases over time but still remains significant at 18 years (Table 4) (112–115). However some studies have not observed the same results and shown a lack of significance as early as six years (116). Despite the decreasing risk of asthma, one study found an increase in respiratory symptoms in 28- 30 year olds who previously had a RSV LRTI and in particular pneumonia. They also found a statistically significant decrease in lung function for this group

(117). Other studies have also shown that rhinovirus or non-respiratory syncytial virus LRTIs have a stronger association with recurrent wheeze/ asthma (118–120). Although viral cause may play a role in wheezing/asthma causation has not been confirmed, based on the evidence to date, this is not likely. This is because by two years most children have had a RSV and or RV infection. Disease severity could be a cofounder and could explain the variation in association between early viral LRTI and recurrent wheeze/asthma. Escobar *et al*., investigated the odds of wheezing and asthma based on RSV bronchiolitis severity. The retrospective study classed severity as no visit, outpatient clinic visit, Emergency Department (ED) visit and hospitalisation. The study demonstrated increasing proportion of asthma diagnoses in an increasing severity dependent manner: No Visit (9 %), Outpatient (16 %), ED (19%) and Hospitalization (22%), ($p<0.001$). The odds of asthma at five years relative to no bronchiolitis visits was calculated with hospitalised infants 2.82 (95% CI 2.61-3.03). There was also a positive correlation between the number of bronchiolitis visits and the risk of asthma (121).

As seen in the studies mentioned in the previous section, atopy and allergic sensitisation play a key role in the development of asthma. It is unclear whether an early infection causes this, or that the risk factors leading to asthma also predispose infants to an early severe LRTI. RV is associated with atopy and allergic sensitization, and Carroll *et al.* showed that infants with a maternal history of asthma were predisposed to have RV infection and maternal asthma increased the severity in RV and not in RSV (122). These differences may highlight the different underlying immune responses to different viruses. Although we do not know if early exposure does cause long-lasting damage that predisposes infants to wheezing/asthma the evidence suggests that these factors enhance the effects

	RSV Group	Control	Relative Risk
3 years: asthma(112)	23%	1%	21.8 (95% CI 2.90 – 163.57)
7 years: asthma (p<0.001)(113)	23%	2%	10.88 (95% CI 2.51-47.11)
13 years: asthma (p-0.001)(114)	28%	3.3%	8.7(95% CI 2.6 – 28.9)
18 years: Current asthma (p<0.001)(115)	33%	7%	

Table 4 Risk of asthma in Sigurs et al.'s cohort over time (112–115)

1.4.8 Aims and objectives of the research undertaken in this thesis

This opening chapter has shown viral causes of respiratory illness significantly contribute to morbidity and mortality in infants and pre-school children. Although in the UK most deaths due viral induced bronchiolitis and pneumonia in children under two years happen in those with other comorbidities, there is a group of otherwise healthy infants that have severe disease who require hospitalisation and critical care support. Unfortunately, we currently do not have any vaccines or curative treatment for these viruses. We are also lacking a fullt validated scoring system to monitor children with bronchiolitis and viral induced wheeze. This is also needed to assess whether new treatments are efficacious.

Furthermore, there is emerging evidence from Europe and the USA that a significant early respiratory viral infection is linked to long term illness such as recurrent wheeze and asthma. Very little research in this area has been undertaken in the UK.

The research outlined in this thesis aims to:

1. Complete the validation of the Liverpool Infant Bronchiolitis Severity Score by assessing responsiveness to change in children under two years with a diagnosis of bronchiolitis or viral induced wheeze
2. To investigate differences in presentation and subsequent respiratory morbidity in infants $\leq$ six months of age who present to A&E with viral acute respiratory infection and a positive RSV or RV respiratory sample

2. Longitudinal Validation of Liverpool Infant Bronchiolitis Severity Score

2.1 Introduction

As part of Dr Clare van Miert's NIHR-funded PhD studentship, the Liverpool Infant Bronchiolitis Severity Score (LIBSS) was developed as a tool to monitor the clinical condition of infants with bronchiolitis. It was validated against the COSMIN checklist which aims to provide a reference for those wishing to assess the measurement properties of a scoring system. To date, reliability, validity and interpretability have been tested, but responsiveness has not been explored. Responsiveness aims to detect changes in the element being measured over time (41). For the LIBSS questionnaire, this is important because the overall purpose of the LIBSS questionnaire is to help identify those at risk of clinical deterioration. To do this, we need to be sure that changes in score over time reflect a change in a patient's condition.

When this MPhil started in September 2016, my original aim was to longitudinally validate the LIBSS questionnaire in infants with bronchiolitis over the 2016/17 bronchiolitis season (Appendix 1). Unfortunately, there were significant delays associated with seeking ethical permission and particularly with getting HRA approval (Figure 5, Appendices 5-7). Although a favourable ethical response was given at the end of December 2016, HRA approval was not in place until March 2017, well beyond the RSV 'season'. During the ethical submission process, we anticipated that we might struggle with recruiting infants with bronchiolitis. Consequently, we changed our study to a pilot on the use of LIBSS in a hospital environment. A pilot study is a type of feasibility study that checks that all parts of an intended study design work together in reality. Data collected from a pilot study can be included in the main study's data analysis and is known as an internal pilot; if data is excluded, an external pilot was carried out (123,124). There are several valid reasons to carry out a pilot study:

1. To assess the feasibility of the processes that are essential to the main study
2. To assess potential resource and time management issues that may occur during the main study

3. To assess human and data management problems
4. To investigate issues to do with safety, dose response and effect and variance of the effect

For these same reasons, we also broadened our inclusion criteria to include viral induced wheeze in children under 2 years of age rather than just infants with bronchiolitis. Furthermore, we decided to validate LIBSS questionnaire responses contemporaneously against Paediatric Early Warning (PEW) score responses.

2.1.1.1 Aims

To assess the feasibility of longitudinally validating the Liverpool Bronchiolitis Score in children under two years with viral induced wheeze or bronchiolitis by:

- Carrying out study as per protocol below to assess: recruitment, consent process and intervention
- Comparing the Liverpool Infant Bronchiolitis Severity Score against the Paediatric Early Warning System
- Collecting or 'scavenging' respiratory samples from participants to investigate biomarkers of severity after recruitment

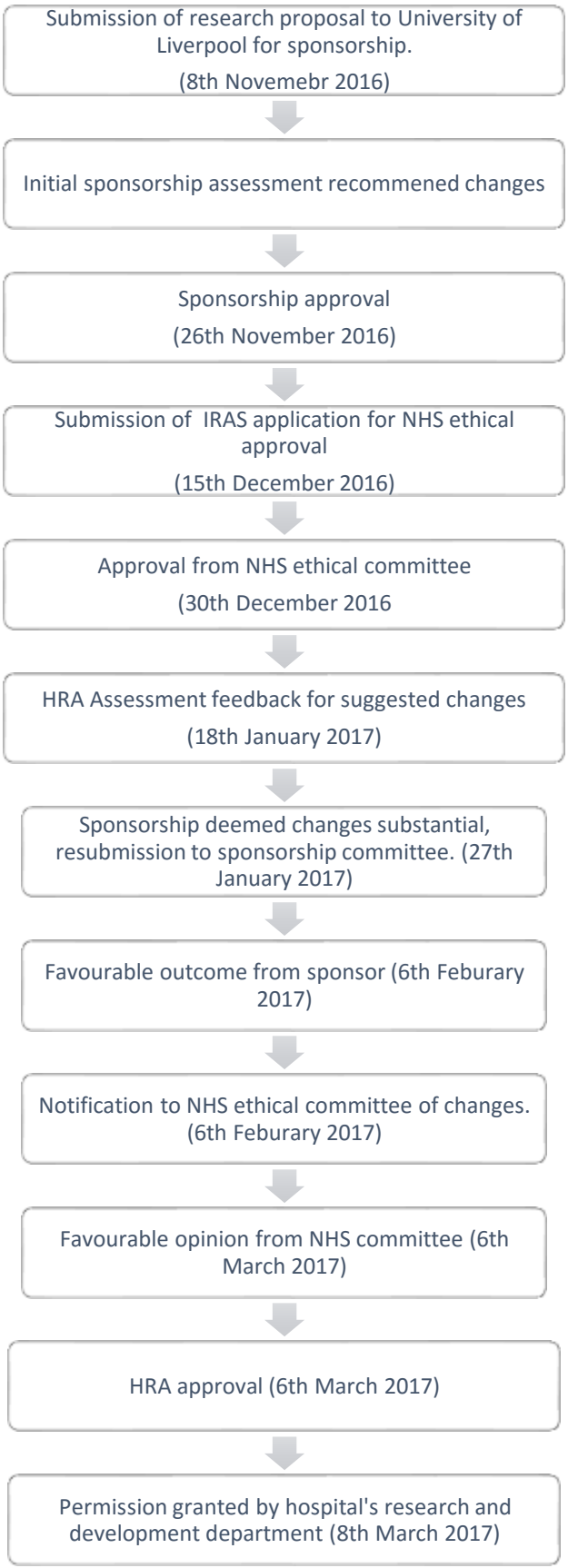

Figure 5 *Flow chart of approval process*

2.1.2 Methods

2.1.2.1 LIBSS

During the study, infants were examined twice daily using the LIBSS by investigator PA. Patient's notes were reviewed to confirm clinical diagnoses and assess the health of the child between investigator visits. Total scores were calculated and participants were categorised based on cut-offs developed during LIBSS validation, i.e. mild < 11, moderate 11 – 20, severe ≥ 21. After data collection and recruitment, investigator PA calculated admission/A&E presentation and discharge scores by looking at electronic case notes and first recorded PEWS on clerking in A&E.

2.1.2.2 Respiratory Samples

If nasal pharyngeal samples were taken by participant's direct healthcare team or suctioning was performed, these samples were 'scavenged' from the laboratory or wards. Samples were taken for potential future work looking for biomarkers that may reflect or predict disease severity.

2.1.2.3 Data collection:

During recruitment, a brief clerking was taken from parents to gather information about the past medical history of the child, and the course of the illness for their current hospital presentation. This information was compared to participant's medical notes for completeness. For every examination carried out by the investigator, parents and nursing staff were asked about the health of the child and notes were checked for any updates or changes in management e.g. changes in oxygen use. All this information was inputted into an Excel spread sheet and resulted in the following headings:

- Admission PEWS and LIBSS
- Discharge PEWS and LIBSS
- Diagnosis
- Co-morbidities
- Oxygen required during hospital stay
- Nasogastric feeding during hospital stay
- Gender
- Age
- Admission and Discharge date
- LIBSS Questionnaire date
- LIBSS Questionnaire answers

2.1.2.4 Recruitment strategy:

PowerPoint presentations about the study were given to General Paediatric and Emergency departments and feedback was elicited on how best to recruit patients within their departments. Staff and research nurses in these departments and HDU were also contacted to raise awareness about the study and gain ideas on the best way to recruit participants.

2.1.2.5 Inclusion criteria:

- Children under 24 months with viral induced wheeze or acute bronchiolitis
- Children with parents/guardians who understand English and have capacity to consent for their child

2.1.2.6 Exclusion criteria:

- Children who have been given palivizumab
- Children with known immunocompromise
- Children with severe underlying cardiorespiratory disease e.g. cyanotic heart disease, CF, severe tracheomalacia and bronchopulmonary dysplasia
- Children with known neuromuscular disorder

2.1.2.7 Data analysis:

Comments received during study by staff, parents and researcher (PA) were incorporated into assessment of feasibility by PA. Analysis of data was carried out on Excel and SPSOS Version 24 and 22. Scores from PEWS and LIBSS were plotted to see the relationship between, and linear categories were further tested for the degree of correlation using Pearson's Correlation Coefficient.

2.1.3 Results

2.1.3.1 Recruitment

Recruitment for this study began on 15th March 2017 for four weeks. Overall twenty families were approached to take part in the study. In total, PA approached 17 parents on the General Paediatric ward to take part in the study and 3 from Emergency Department Observation Unit (EDU) (see Figure 6). Only two families refused to take part, another two participants were excluded due to underlying medical conditions (phenylketonuria and one with severe congenital respiratory abnormalities which meant they regularly required oxygen). One child's diagnosis changed during participation so data were not analysed. Consequently, fifteen participants were included in analysis with twelve recruited from the General Paediatric ward and three from EDU. During the study, no children were recruited from HDU, although one patient was admitted there overnight prior to recruitment. Overall PA carried out 32 LIBSS examinations.

The time between A&E presentation and recruitment ranged between 0 – 4 days (Figure 7). 5/15 (33%) of patients were recruited within the first 24 hours of admission and 14/15 (93%) within the first 48 hours. The participant that was recruited on the fourth day was admitted straight to HDU and was then transferred to a surgical ward as this was the only available bed. There are several reasons why there were delays in recruitment. As I was not part of the admitting clinical team, I was not allowed to search for potential recruits through the hospital computer system and had to rely on wards contacting me, either in person when I was on the wards or by phone. Consequently, potential participants not on general medical wards were not recruited. I received no phone calls during the study from any ward. I recruited Monday – Friday between 9 – 5, but most wards preferred me to attempt recruitment after ward rounds had been completed (~10:30 am). Children that presented during the weekend or the evenings were not seen until I attended the wards on the following Monday.

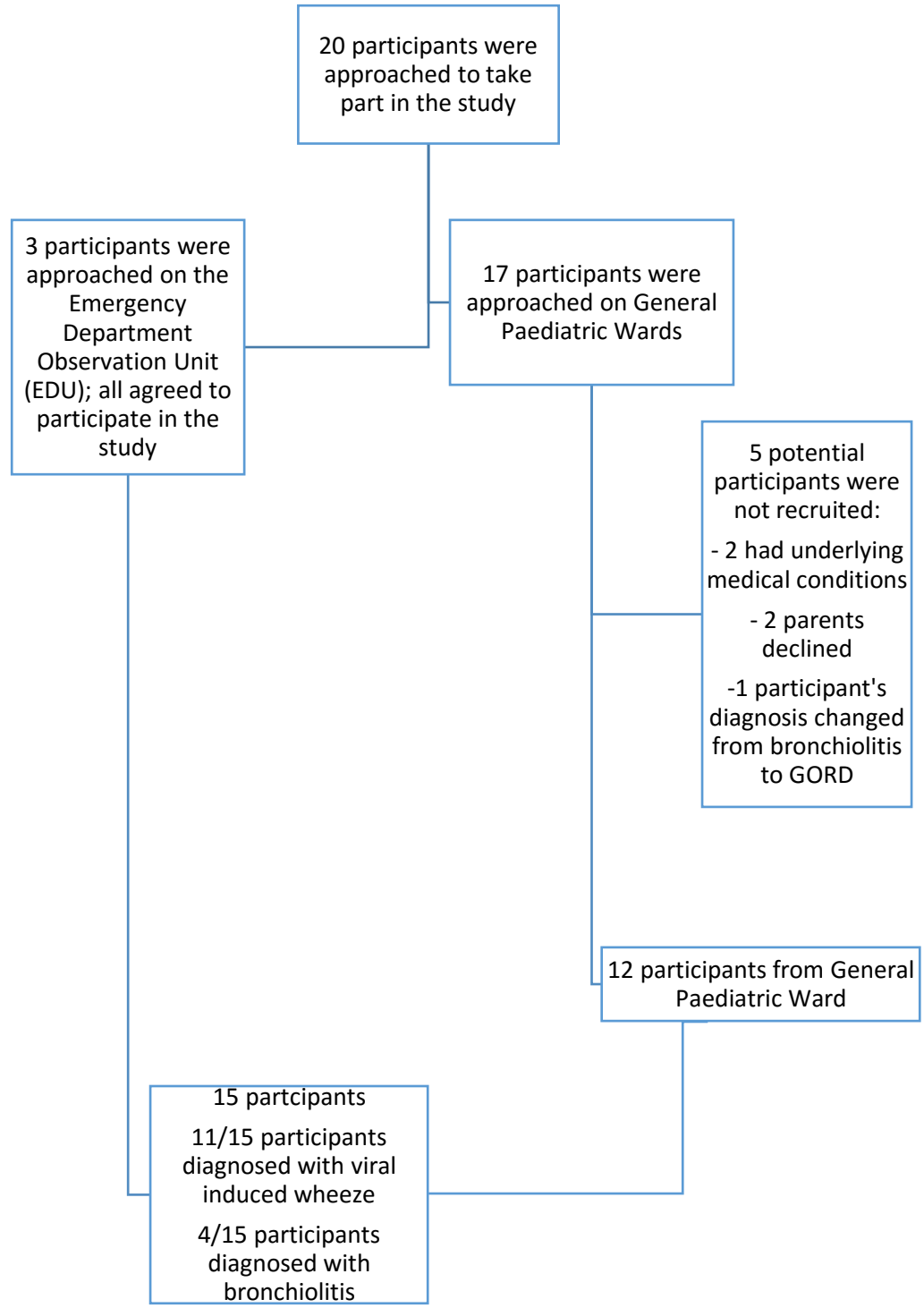

Figure 6 Recruitment Flow Chart

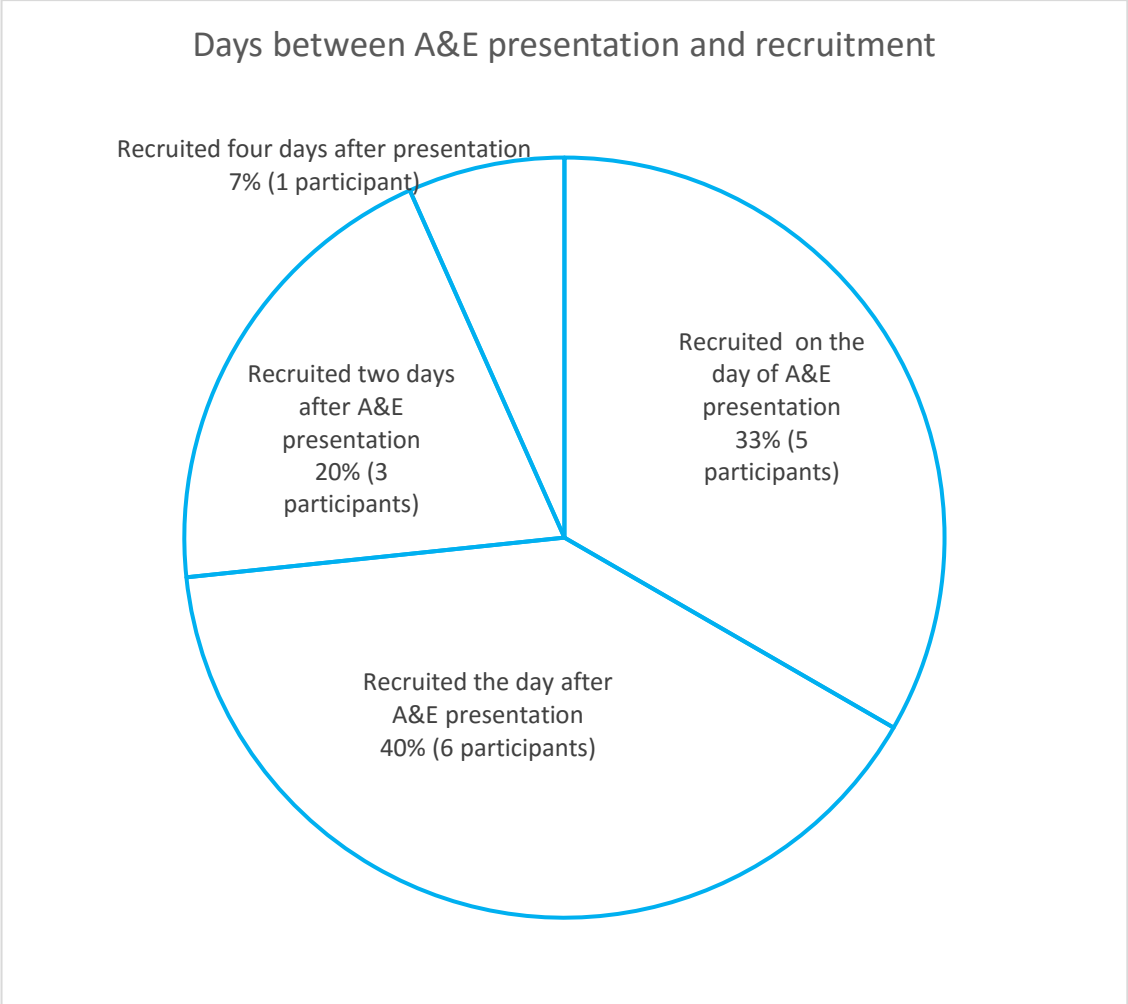

Figure 7 Pie chart of time taken to recruit participants

2.1.3.2 Consent

There were two parts to the consent form. Parents could consent to only having their child assessed using the LIBSS, or they could consent to a LIBSS assessment and additional respiratory samples taken. 100% of parents who agreed to be involved in the study consented for their child to be assessed using the LIBSS, but only 60% (9/15) of parents consented to additional samples being taken. Three parents were happy with a potential NPA if their child's respiratory symptoms increased LIBSS.

2.1.3.3 LIBSS

The mean number of LIBSS completed was two, however this was only done in seven children (see table 5) and the overall mean length of stay was two days. Figure 8 shows the mean total LIBSS on presentation to A&E/admission and questionnaires completed in real time by PA, this is plotted with the mean day of illness for the corresponding time. Figure 9 shows the same for the total PEWS scores.

On admission, the mean day of illness was 6. This rose to 7.06 days for the first set of LIBSS carried out. The number of sick days was calculated by asking parents when they first noticed their child's illness. The range for the number of sick days was 0-40 days, one parent felt that their child had not recovered from their illness a six weeks earlier. This participant attended A&E on the original occasion but was not admitted. The second questionnaire saw a fall in the mean day of illness due to the fall in recorded scores due to discharges. The two participants with the longest length of illness were a part of the group that were discharged.

There is some evidence that the LIBSS is responsive to changes in health status. The mean LIBSS was highest on admission and 0 on discharge. The PEWS follows the general trend of high scores on admission and low scores on discharge. Unlike the LIBSS, not all scores were 0 on discharge. Four children had a PEWS score of 1: two had this score because their oxygen saturation was 92%, another child was asleep and one child had a respiratory rate just below the normal limits for their age. A PEWS score between 0 and 2 is normal variation and does not require further investigation so the variation in score did not delay this discharge.

In addition to this participant 15 first questionnaire was taken just before and the second after nebulisers, this showed a reduction in score, the changes were seen in the child's respiratory rate and effort of breathing, on the LIBSS score changed from 6 to 0 and for PEWS 3 to 0.

Participant Number	Admission Data	Questionnaire 1	Questionnaire 2	Questionnaire 3	Questionnaire 4	Questionnaire 5	Questionnaire 6	Discharge Data
1	X	X						X
2	X	X						X
3	X	X	X	X	X	X	x	X
4	X	X	X					X
5	X	X						X
6	X	X	X					X
7	X	X	X	X	X			X
8	X	X						X
9	X	X						X
10	X	X						X
11	X	X	X	X				X
12	X	X						X
13	X	X	X	X				X
14	X	X						X
15	X	X	X	X	X			X

Table 5 Number of questionnaires per participant (admission and discharge information was collected retrospectively)

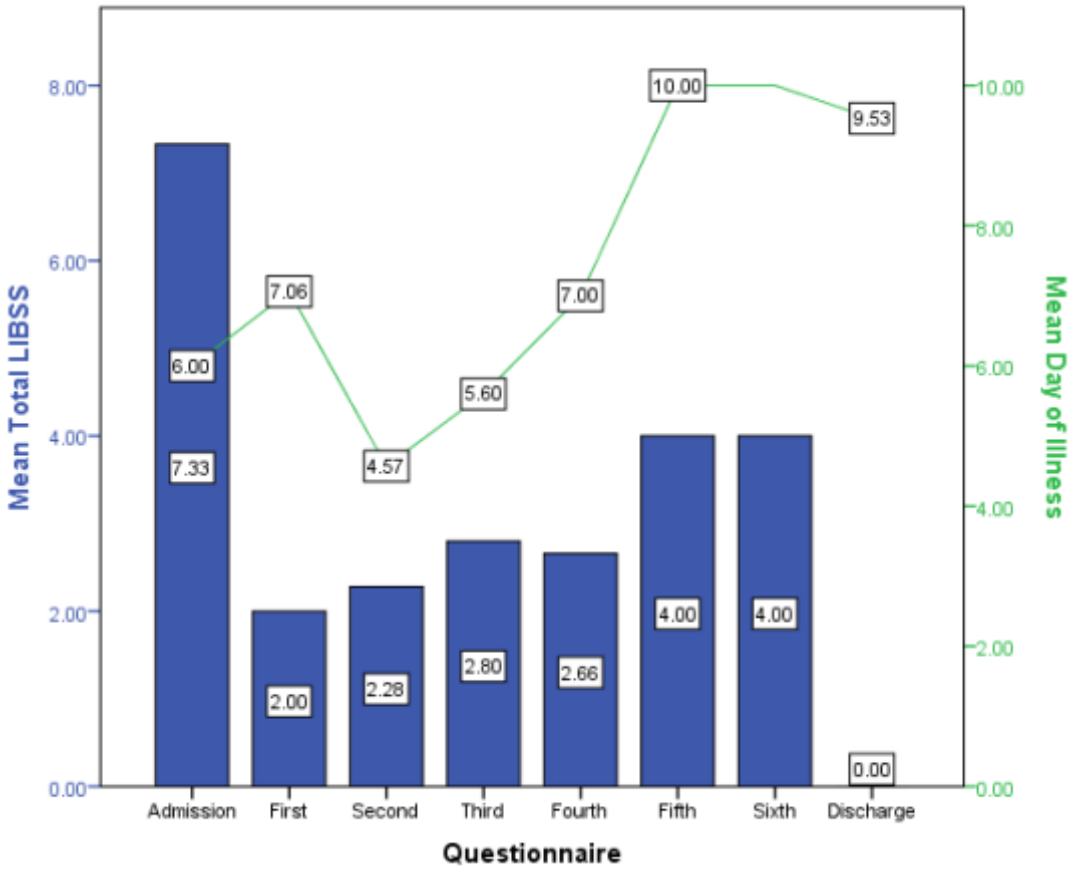

Figure 8 Mean total severity scores for participants based on LIBSS and mean day of illness

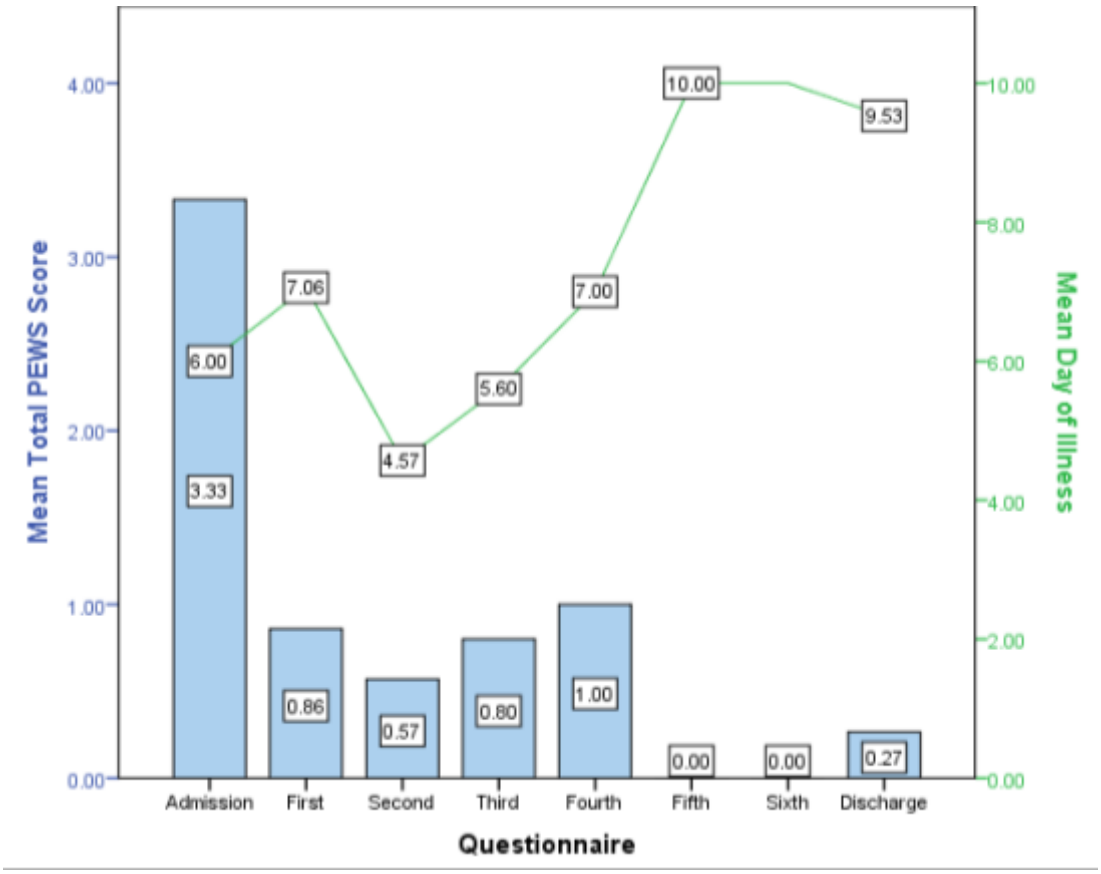

Figure 9 Mean total PEWS scores and mean day of illness

2.1.3.4 Participant Characteristics

The mean age of participants was ten months (uncorrected for prematurity) with a range from less than 1 month to 19 months (Figure 10). The cohort was split relatively evenly with 7 girls and 8 boys and, 4/15 patients were diagnosed with bronchiolitis and 11/15 with viral induced wheeze.

Eight of fifteen participants had no predisposing risk factors for ARI. Of the remainder, six children had a personal or family history of recurrent wheeze, atopy or allergic sensitisation, with two of these infants having at least one parent who smoked. One child was a premature twin that required special care at birth.

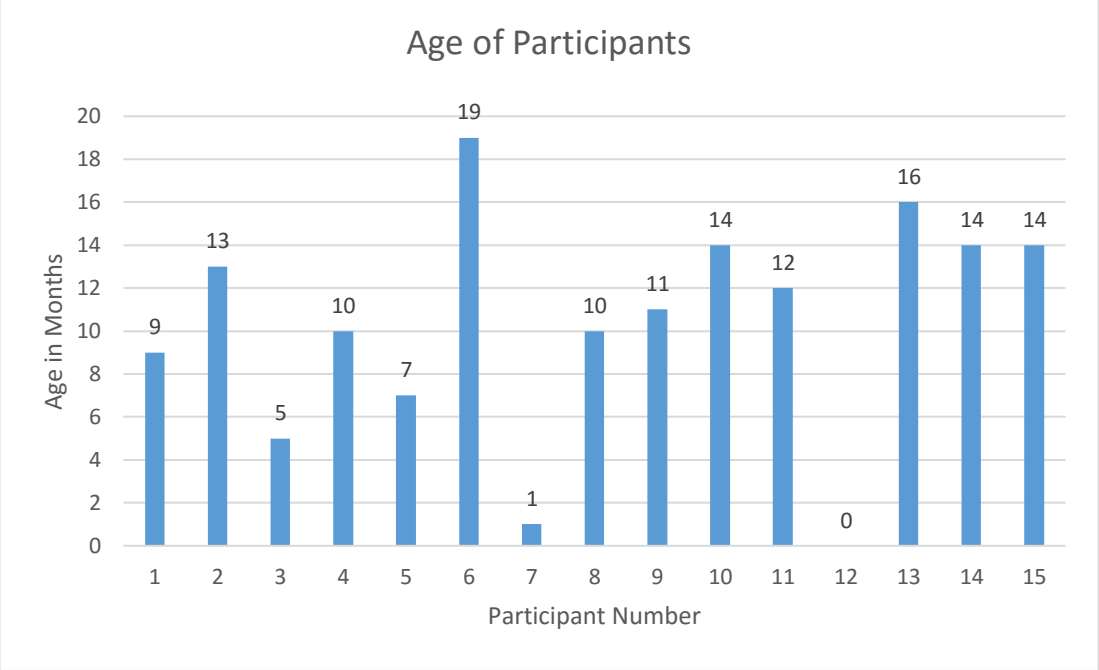

Figure 10 Age of participants

2.1.3.5 Severity Scores

Responsiveness was assessed by looking at the correlation in severity scores for LIBSS and PEWS. LIBSS total on hospital presentation varied, 11 children were categorised as having mild disease, 3 as having moderate disease, and 1 with severe disease (LIBSS score of 28). PEWS scores ranged from 0 -7. LIBSS and PEWS scores on admission correlated strongly with a correlation coefficient of 0.864 ($p<0.001$) (Figure 11 and Table 6). Looking at scores from the first questionnaire following admission, they varied from 0 – 10, and PEWS scores of 0 – 4. Correlation between LIBSS and PEWS scores on first questionnaires was highly positive (PCC: 0.845; $p<0.001$). Seven participants had a second questionnaire recorded with a range of scores from 0 – 8 and PEWS scores of 0 – 2. There was decrease in correlation but significance remained (0.786; $p=0.036$). Correlation was still seen in children who required nasogastric feeding even though this was not a part of the PEWS (PCC: 0.950; $p<0.001$)

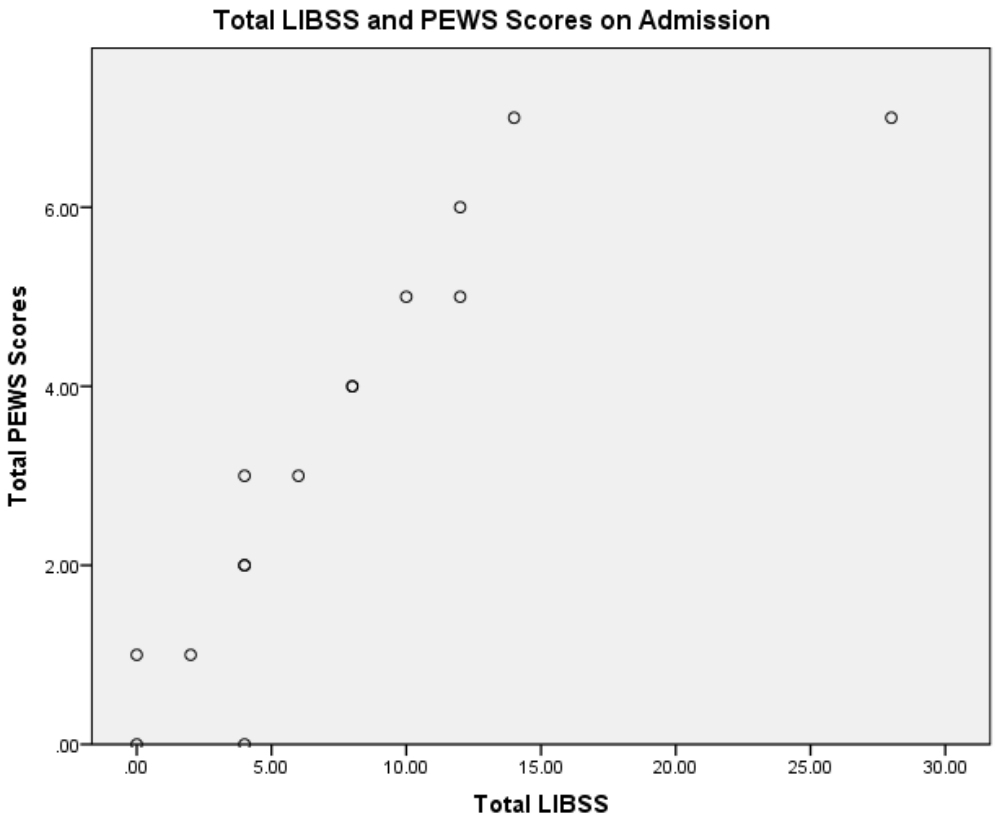

Figure 11 Scatter graph of LIBSS and PEWS Scores on Admission

Table 6 Pearson's Correlation Test for PEWS Scores versus LIBSS

	Pearson's Correlation Coefficient	p value	Number of Participants
Admission	0.864	<0.001	15
First Questionnaire	0.845	<0.001	15
Second Questionnaire	0.786	0.036	7
Third Questionnaire	0.848	0.070	5
Additional Feeding Questionnaires	0.950	<0.001	11

2.1.3.6 Respiratory samples

Only four samples were scavenged from laboratories. This low number of respiratory samples collected and analysed reflects the fact that routine respiratory viral PCR testing stopped on the 28th February 2017 and that many participants were over six months and presented with clear diagnosis of viral induced wheeze.

2.2 Discussion

The original aim or this study was to longitudinally validate the LIBSS in infants with bronchiolitis and collect or scavenged respiratory samples for a predictive biomarker testing in the future. However, because of difficulties with the ethics/HRA process, the aims of this study were revised to assessing the feasibility of longitudinally validating the LIBSS in children under two years with viral induced wheeze or bronchiolitis. The LIBSS was generally quick and easy to complete. Most children were recruited on general medical wards and categorised according to LIBSS as having mild disease. Over a one month period, 20 parents were approached and 15 children were recruited bronchiolitis/viral induced wheeze; 33% (5 children) of children were recruited on the day of presentation and 40% (6 children) on the following day.

The most effective way of recruiting patients was by asking staff for any eligible patients in person. It is not surprising that no phone calls were received from staff as they were busy and contacting researchers was understandably not a priority. Frequently visiting wards meant that staff became more aware of the eligibility criteria and mentioned potential patients coming up to EDU or General Paediatrics. The time taken between patient presentation and recruitment was poor. Data from presentation is particularly important for developing a predictive element or escalation plan for the LIBSS. The observation period was limited to weekdays and work hours, but children that present at night or on weekends may be sicker as parents may feel they cannot wait until the next day or the week days for a medical assessment. Some children had a prolonged stay as they were unable to maintain oxygen saturations above 92% whilst sleeping and we have missed this data. This information is important as it is a confounding factor for infants scoring 0 during LIBSS assessment in the day but not being discharged. Another use for this data is looking at any associations between otherwise healthy children who cannot maintain the oxygen saturations in sleep and biomarkers.

Eighty percent of parents approached agreed to participate in the study. This consent rate may lessen for sicker and younger children as parents may be more nervous and not want the added stress of participating in a study. 40% (6 children) of participants did not consent to additional samples being taken, although this number may increase during bronchiolitis season especially amongst sicker children as they are more likely have respiratory secretions. These figures suggest parents did not have a problem the LIBSS questionnaire, but may have issues with NPA samples. Parents' attitudes towards NPAs especially in children with milder

disease is understandable as this may be the first time they had heard of this kind of sampling and may find it too invasive. Although attitudes were understandable, there will be difficulties in obtaining NPAs in children with milder disease therefore finding cut-off points for a potential biomarker will be difficult.

The LIBSS was easy to use after some training prior to recruitment with CvM, creator of LIBSS. The score itself has all the components of a paediatric early warning score with exception of temperature, so if user is familiar with a PEWS assessment or a paediatric respiratory examination there should be no difficulty. The time taken to carry out the LIBSS is similar to the PEWS so should not affect carrying out this study on a larger scale. One issue for the future is that PEWS are currently recorded electronically at Alder Hey, and nurses write down information and input information into computers. Although the LIBSS has 10 questions, for some questions there are multiple options and it may not be possible to remember the finer details and write down the points for a later date. So, for now LIBSS will have to remain in paper form.

In this cohort, one of the participants was severely ill and this was on A&E presentation. The majority had a mild illness and only two children had moderate illness. This may be due to the age of participants as on average they were older than the peak bronchiolitis age of six months and better able to cope with respiratory illness. Secondly, most participants had viral induced wheeze and may only have mild symptoms between nebulizers/inhalers. One of the participants that was considered to have moderate symptoms was seen soon after they arrived in EDU and prior to medication, but after medication their symptoms were mild.

Over the recruitment period, the mean number of questionnaires completed per participant was 2, with a mean LIBS score of 2. However, only 50% of those recruited had a second questionnaire completed which may affect the precision of responsiveness observed, looking at admission and discharge data retrospectively may help combat this as LIBSS can be calculated by looking at electronically recorded clinical notes, PEWS and fluid charts. However, the accuracy is diminished as there is no guarantee that the healthcare professional will input information about urine output and appearance of the child. From the results, we can interpret that there was a strong positive correlation between PEWS scores and LIBSS scores on admission, first questionnaires but not on discharge due to a non-linear pattern. Despite PEWS scores not incorporating a feeding element, a strong positive correlation was seen in those who had a score for feeding and the matching PEWS. From the overall results, we can see that LIBSS scores in milder children correlate to PEWS scores.

However, it is not clear how well this correlation will hold up in children with severe respiratory symptoms where EOB in PEWS maximum score is 2 versus 20 in the LIBSS. There is some indication that the LIBSS changes with health status all children were discharged with a score of 0. Mean day of illness and scores were affected by sample size and show a need for a greater sample size. The non-linear results could also reflect the true hospital population. On admission scores will be high as no intervention is given, fortunately most children respond well to intervention and will only require a few days in hospital. The increase in severity score on the fourth set of questionnaires reflects the analysis of the select children that have a severe illness and require a higher level of care and a longer hospital stay.

Duarte *et al.* recruited healthy children on their first bronchiolitis hospitalisation, which was determined by a clinician not involved in the study. The Modified Wood's Clinical Asthma Score (M-WCAS) was compared to the Tal *et al*. severity score as it had undergone validation. The M-WCAS was assessed for construct validity, inter-rater agreement, sensitivity to change (responsiveness). Overall 54 patients were recruited for the study with a median age of 5 months. There was a positive correlation between the two scoring systems (Spearman's Correlation Coefficient = 0.761, $p < 0.001$). Responsiveness was assessed by comparing scores on admission to medical ward and discharge scores, results showed a significantly higher admission score 2.5 (1.9–2.5) vs. 1.0 (0.5–1.6), $p < 0.001$(38). This study had a similar sample size to our minimum recruitment target, they calculated their sample size based on the guidance of Walter *et al.* (125). Like our study the M-WCAS showed a positive correlation to another score quantifying severity by symptoms which is the preferred method of assessing responsiveness according to the COSMIN checklist (41). It is questionable whether Tal's severity score was the gold standard at that point in time as it was not widely used in clinical practice and content validity or responsiveness had not been tested (37).

Fernandes *et al.,* looked at the responsiveness of the RDAI and RACS in infants diagnosed with moderate bronchiolitis attending A&E across Canada. Using respiratory rate reduction and probability of admission as their anchors of change between improved versus stable/deteriorated patients ROC analysis and standardised effect size were calculated. RDAI and RACs were both below the cut-off for acceptability (0.64 and 0.65) (35). ROC analysis was an approved choice by the COSMIN in the assessment of responsiveness (41). Although Fernandes *et al*. carried out ROC analysis, their cohort was in moderately ill infants on their first bronchiolitis episode so we cannot be sure of the responsiveness in all hospitalised infants with bronchiolitis and perhaps including different types of severity will change the

ROC calculation. The RDAI had previously shown poor short term responsiveness when tested by Destino *et al.* in 29 infants with bronchiolitis. The RDAI was a poor discriminator of those who discharged and admitted based on 195 infants scores and inter-reliability was also poor (tested in 65 infants). Destino's group investigated responsiveness by scoring children before and 15 minutes after intervention. In these infants the RDAI had a weak positive correlation to the Children's Hospital of Wisconsin Respiratory Score (CHWRS) (r=0.39 p=0.04) (30). All three studies have shown it is possible to assess how responsive a severity score is in bronchiolitis.

2.2.1.1 Strengths

This study has a number of strengths:

1. Assessing the feasibility of longitudinally validating LIBSS scoring system, we have gained some useful information on how to conduct a definitive validation, namely that our current recruitment strategy works well for capturing participants on General Paediatric wards but more time is also needed to decide how children in A&E will be assessed.
2. The LIBSS was compared to the current surveillance tool used in clinical practice (Paediatric Early Warning System)
3. Most scoring systems for bronchiolitis tend to be validated as they are being used in clinical trials. Validation of LIBSS will be undertaken separate to any clinical trial.
4. Despite recruiting at the very end of RSV season, we still managed to recruit some patients with bronchiolitis whose symptoms and signs varied based on day of illness and severity.
5. Although the study sample size was small, the cohort was large enough to highlight flaws in study design and recruitment process. It also highlighted the practicalities of recruiting multiple patients contemporaneously. On one day, 5 new participants which gave a realistic idea of what might happen during the height of the RSV season.

2.2.1.2 Limitations

As expected, the LIBSS was not definitively validated. There is also difficulty in generalising data, as the LIBSS was not been designed for children over one years or for those with viral induced wheeze. To test true responsiveness in children with viral induced wheeze, before

and after bronchodilator, scores are required as they have a known effect on symptoms. Although viral induced wheeze is similar to bronchiolitis in some respects there are still differences such as the peak age (12). The mean age of our cohort was 10 months, children at this stage may be showing signs of recurrent wheeze and this is seen in our participant with day of illness of 40. The child most likely has the beginning of what could be asthma or multiple trigger wheeze (47). In a larger cohort analysing those with a diagnosis of bronchiolitis separately is necessary to test true responsiveness. Only four participants in this study had a NPA to scavenge and no children had a NPA taken. This puts further pressure on the next year to collect specimens for biomarker investigations. Although in this study we have compared LIBSS to PEWS, the latter, while proven to reduce the likelihood of mortality in PICU, has not undergone any formal psychometric testing (126).

2.2.1.3 Recommendations

A definitive validation of LIBSS needs to be undertaken in infants with bronchiolitis. This will hopefully be done by myself and others this coming winter. In future, it is possible that LIBSS may become a useful tool for assessing older children with bronchiolitis (particularly given that the recent NICE guidelines have extended the age at which children can be diagnosed as having bronchiolitis from 12 months to 24 months and possibly viral induced wheeze(12).

Recruitment and data collection could be improved by increasing manpower, for example by placing researchers on both the wards and in the emergency department. Consent processes could then happen early during admission after initial assessment and management of child. Although the LIBSS has cut-off scores for severity, unlike the PEWS there is no guidance for escalating care and this needs to be investigated further.

2.2.1.4 Conclusion

This study showed that it is indeed possible to assess the responsiveness of the LIBSS, however further work to study protocol will need to be done to recruit enough patients for the definitive study. In our cohort, the LIBSS correlated to PEWS scores, however our study limitations mean that further recruitment and more longitudinal data for individuals is required before biomarker investigations and responsiveness can be tested as recommend by COSMIN guidelines.

3. Respiratory Morbidity after an Early RSV or Rhinovirus Infection

Early lower respiratory tract infection caused by RSV or RV is associated with recurrent wheeze and asthma development (110,115,120,127) but a causal relationship between viral infection and recurrent wheeze/ asthma has not been established. Other factors may contribute such as disease severity of LRTI, a personal or familial history of allergic sensitisation and atopy. Looking at the initial infection characteristics may help identify distinctive features of illness based on infective virus and how they may contribute to long term respiratory health.

3.2.1 Aims

The aims of the work undertaken in this chapter were to:

- Investigate, in a cohort of children ≤ six months of age (uncorrected for prematurity) attending hospital for the first time with viral respiratory tract symptoms, differences in clinical or demographic characteristics associated with hospitalisation with either RSV or RV infection
- Investigate, in the same cohort of children, the relationship between dichotomous factors associated with the first admission to hospital (listed below) and time taken to attend hospital again with respiratory tract symptoms.
    - RSV or RV infection
    - Requiring oxygen during first visit
    - Supportive feeding or fluids
    - Critical care during first hospital stay
    - Age at first presentation
    - Length of stay

3.2.2 Methods

3.2.2.1 Audit registration

In September 2016, the chief investigator (PMc) registered a service evaluation of respiratory admissions to hospital in young infants with the audit department at Alder Hey Children's Hospital (Appendix 8). This service evaluation aimed to determine the clinical and

demographic characteristics of infants under six months of age admitted to hospital with respiratory disease and find out whether any characteristics were associated with subsequent re-attendance to hospital.

3.2.2.2 Respiratory viral PCR testing data

As part of this service evaluation, a laboratory technician (CG) based within the microbiology department at Alder Hey Children's Hospital provided an excel spreadsheet of all respiratory PCR test results between 30st September 2013 - 1st October 2016. This time period was selected as routine viral testing was carried out on respiratory samples. The FilmArray PCR machine, manufactured by BioFire, was used for testing the following pathogens:

- Adenovirus
- *Bordetella pertussis*
- *Chlamydophila pneumonia*
- Coronavirus (229E, HKU1, NL63 and OC43)
- Influenza A H1 2009
- Influenza B
- Human Metapneumovirus
- *Mycoplasma pneumonia*
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory syncytial virus
- Human Rhinovirus/ Enterovirus

This spreadsheet included the following information:

- Alder Hey number
- Age of child
- Date of test
- Specimen Number
- Sample type
- Result
- Department that requested test

3.2.2.3 Demographic and clinical data

Using the respiratory PCR spreadsheet above, the Alder Hey audit department (SS) extracted the following information from the hospital's electronic records system (Meditech*) based on the AH number:

- Date of Birth
- Postcode
- Dates of arrival in Alder Hey A&E of first attendance and all subsequent attendances
- Reason(s) for visit (presenting complaint)
- Clinical diagnosis
- Triage category

* Up until April 2015, data was entered onto the Meditech 5 system. From May 2015, Meditech 6 was used in anticipation of the hospital move from the old to the new Alder Hey.

3.2.2.4 Combined respiratory PCR, demographic and clinical data

A new spreadsheet comprising key domains within the respiratory PCR testing spreadsheet and patient A&E attendance spreadsheet from the audit department was created using the VLOOKUP function in Excel. The combined data were exported into SPSS for recoding and analysis. The SPSS database included the following variables:

- Alder Hey Number
- Date of Birth
- Postcode
- Arrival Date in A&E and for all other visits to A&E
- Reason for visit (presenting complaint)
- Clinical diagnosis/impression
- PCR Result and date

3.2.2.5 Selection Criteria

The following criteria were used to select patients for our service evaluation:

1. Infants $\leq$ six months of age (183 days) with a Liverpool postcode

2. First attendance to A&E/admission to hospital for respiratory tract symptoms (either upper or lower)

3. Respiratory PCR result date within four days of attendance to hospital/admission date

4. Single infection with either RSV or RV on respiratory viral PCR panel. Co-infections were included provided it was not a RSV/RV combination

5. Second hospital attendance for respiratory tract symptoms (either upper or lower) was ≥ 14 days and ≤ one year (365 days) following discharge date for first episode to allow patients across the three years to be analysed together.

3.2.2.6 Additional Information about First Hospital Visit

Additional information about first admission extracted from Meditech by PA, including duration of hospital stay, use of oxygen, nasogastric feeding, IV fluids, antibiotics, PICU or HDU stay. Additional variables were dichotomised using SPSS. A critical care admission was defined as the use of CPAP or HDU or PICU admission during hospitalisation. Those who required nasogastric feeding or intravenous fluids were categorised as requiring supplementary feeding or fluids.

3.2.2.7 Statistical Analysis

SPSS Version 22 and 24 were used to carry out all statistical analysis. Clinical and demographic differences between RSV and RV groups were assessed using Chi-squared analysis. For continuous variables, data were tested for normal distribution. Q-Q plots and Kolmogorov-Smirnov tests revealed that age, and duration of hospital stay were not normally distributed and so non-parametric tests such as Mann-Whitney U tests were used.

The relationship between dichotomous clinical and demographic variables such as virus and oxygen use during first admission, and time to second re-attendance was analysed using Kaplan Meier plots and Cox-regression to determine which variables affect time to re-attend.

3.2.3 Results

Between 30st September 2013- 1st October 2016, 1763 infants ≤ six months (183 days) had a respiratory sample taken for PCR testing at Alder Hey Children's Hospital (Figure 12). 896 had a Liverpool postcode (L1-L38) and were attending hospital for a respiratory cause (867 infants excluded). A further 126 were removed as their A&E/hospital arrival date and PCR result date were not within 4 days if each other. 186 infants were removed as they did not

have a positive RSV or RV NPA. A further 104 were removed for having a RSV/RV coinfection, this left 480 infants had only RSV (but not RV) or RV (but not RSV) detected. One patient was excluded as they were hospitalised throughout the entire 3 year study period. Overall there were 80 coinfections in the final 479 patients, 46 in the RSV group and 34 in the rhinovirus group.

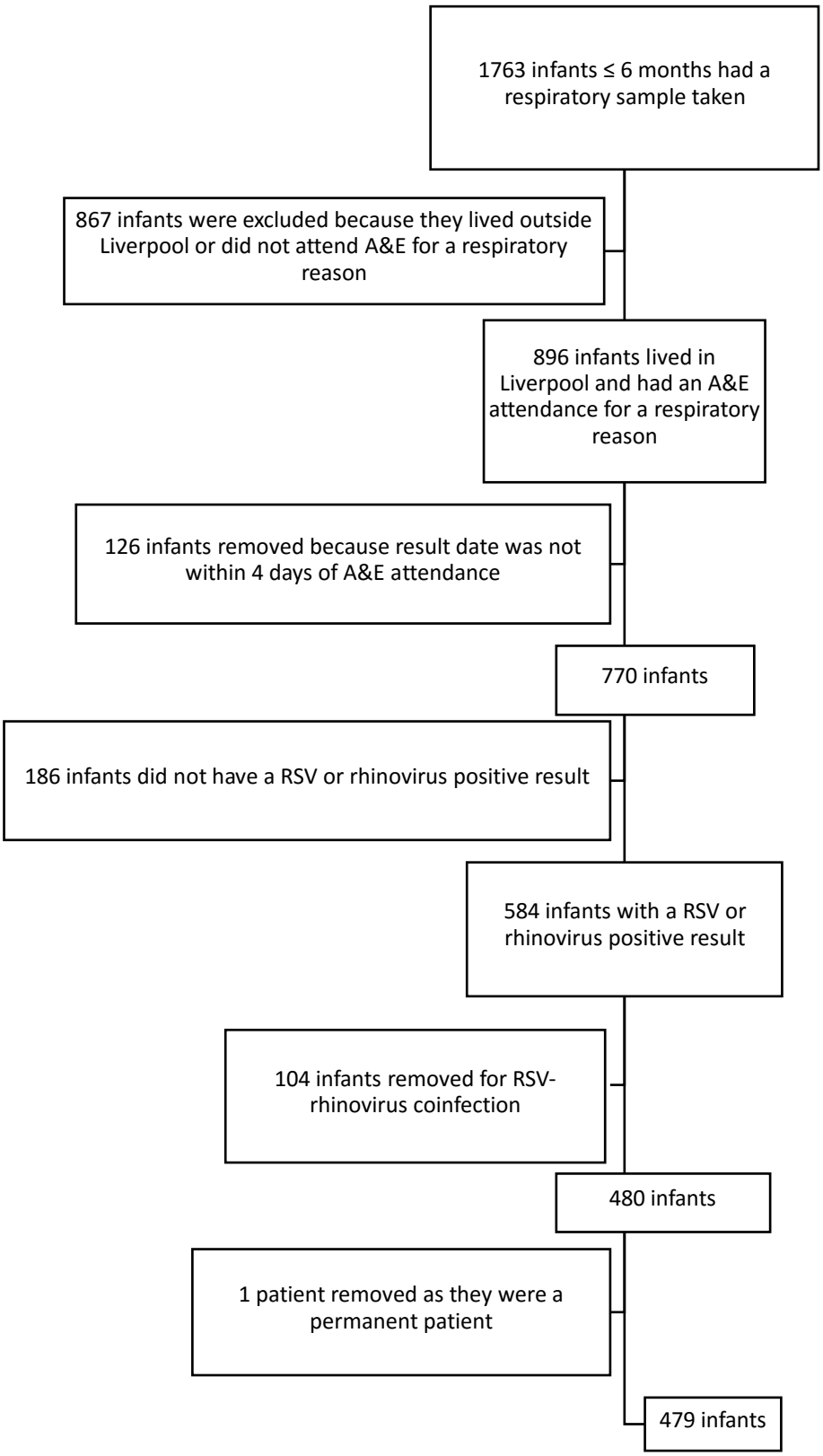

Figure 12 Patient filtering process

3.2.3.1 Patient Characteristics

Table 7 shows the characteristics of each group at first presentation. 360 infants presented with respiratory symptoms and a RSV infection, and 119 infants presented with respiratory symptoms and a RV infection.

At first presentation, there were no differences between infants with RSV or RV infection based on age or number admitted to hospital. However, infants with RSV infection stayed significantly longer in hospital during their first admission than those with RV infection (4 vs 3 days; p=0.01). The RV group had a statistically significant higher proportion of boys (68% vs. 55% p=0.01). Overall the distribution of male to female was 1.4:1, there was a higher proportion of males that were admitted to hospital 59% versus 41%, however this difference did not reach statistical significance (p=0.4).

	RSV Group	*Rhinovirus Group*	*p-value*
Number (Female)	360 (161)	119 (38)	0.01
Number Admitted to hospital on A&E attendance (%)	282 (78)	95 (80)	0.7
Median (IQR) Age (months)	2 (2)	1 (3)	0.6
Median (IQR) Length of hospital stay (days)	4 (4)	3 (4)	0.01

Table 7 Patient demographics

3.2.3.2 Co-infection

Of the 360 infants presenting with respiratory symptoms and RSV infection, 46 (13%) had another non-RV pathogen detected (Table 8). Of the 119 infants presenting with respiratory symptoms and RV infection, 34 (29%) had another non-RSV pathogen detected. Co-infection was significantly more common in the RV group (χ^2 test, $p<0.001$). A particularly interesting finding was 7 patients in the RV group were infected with pertussis infection. However, exclusion of this group did not change the significant findings of this study.

	RSV (n=360)	Rhinovirus (n=119)
Adenovirus	10	5
Bordetella pertussis	0	7
Coronavirus	11	8
Influenza A	2	2
Influenza B	0	1
Human Metapneumovirus	9	4
Mycoplasma pneumoniae	2	0
Parainfluenza 1	2	1
Parainfluenza 2	3	2
Parainfluenza 3	1	2
Parainfluenza 4	6	2
Total	**46 (13%)**	**34 (29%)**

Table 8 Co-infections by participant group

3.2.3.3 Disease severity

Disease severity on first admission to hospital was assessed using several different surrogate measures such as oxygen administration, nasogastric tube feeding, and need for critical care.

Figure 13 shows the number of infants that required oxygen in each viral group. In the RSV group, 271/360 (75.3%) required oxygen, whereas only 61/119 (51.3%) infants in the RV group required oxygen ($p<0.01$ [χ^2 test]). A similar trend was seen when looking at the number of participants that required nasogastric feeds or supplementary intravenous fluids (Figure 14); 249/360 (69.2%) of the RSV group required supplementary feeds/fluid, compared to only 57/119 (47.9%) of the RV group ($p<0.01$ [χ^2 test]). Mann-Whitney tests were carried out to see if there was a correlation between the use of oxygen or supportive feeding and age; both variables were not affected by age ($p=0.1$ and $p=0.9$ respectively).

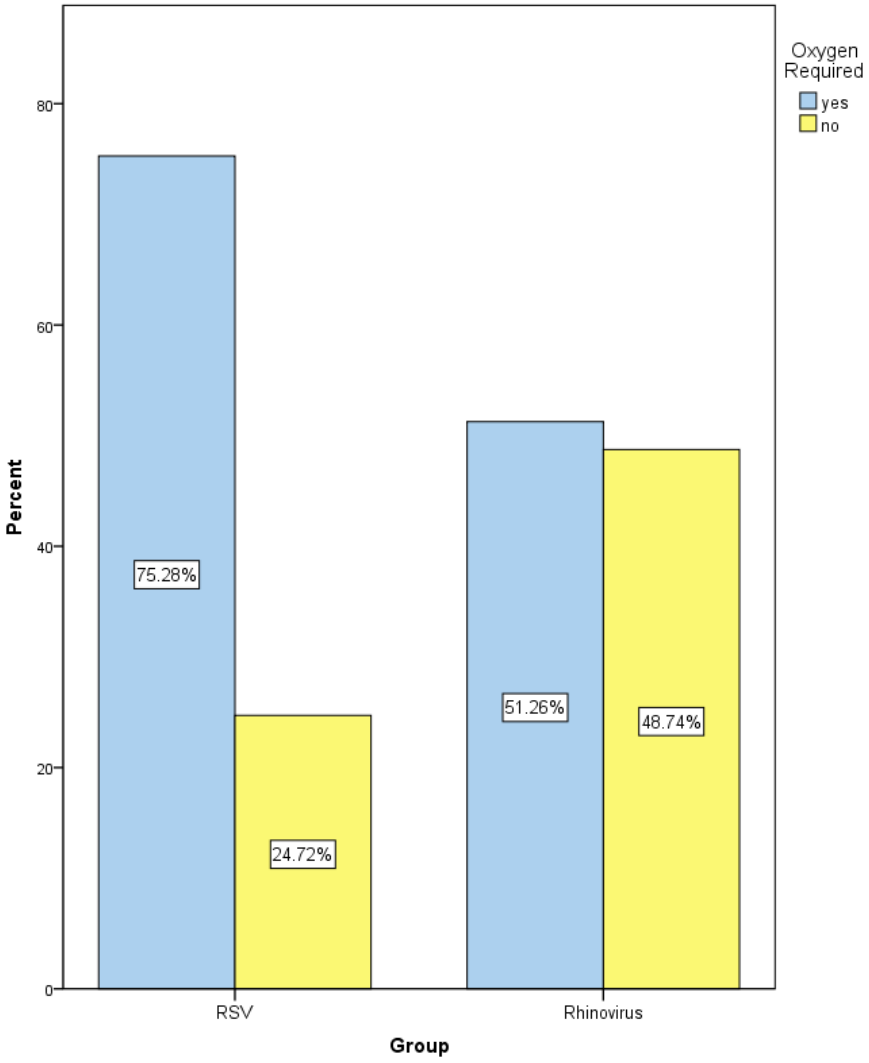

Figure 13 Graph of oxygen use during first hospital visit

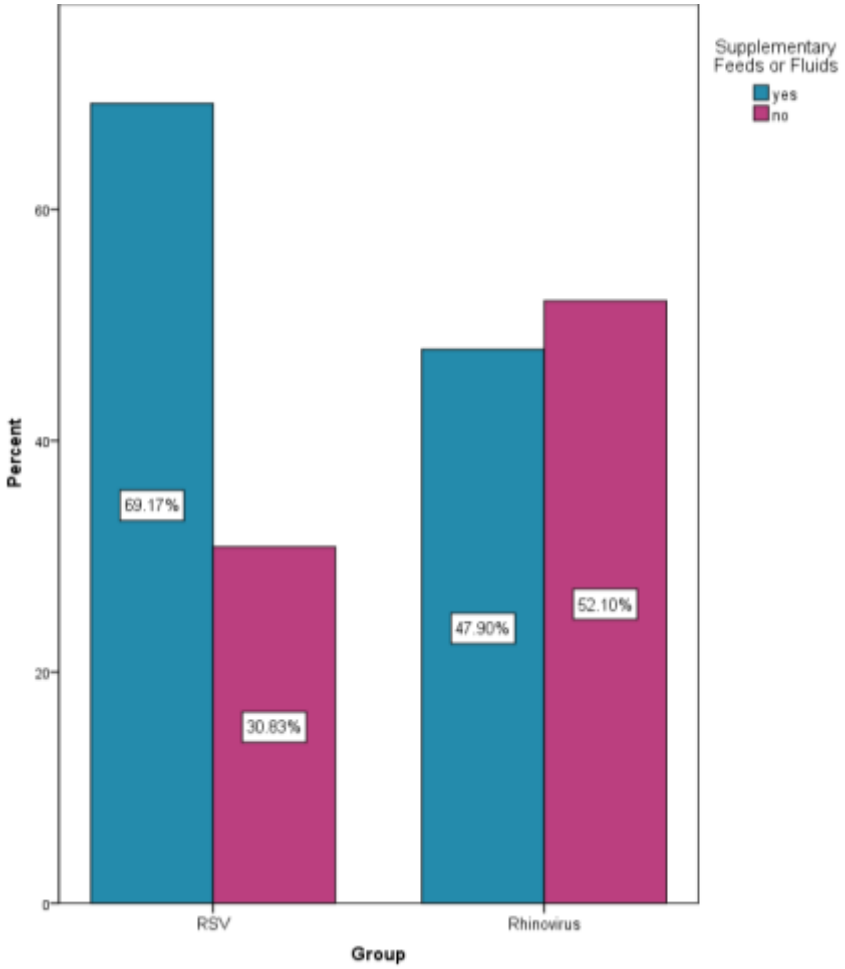

Figure 14 Graph of supplementary feeds or fluids during first hospital visit

Critical care was defined as a need for admission to HDU/PICU or CPAP. In total 68 participants required critical care (Table 9). A greater percentage of patients infected with RV (19.3%) than RSV (12.5%) required critical care but this difference did not reach statistical significance (p=0.06).

	RSV (%)	*RV (%)*	*Median (IQR) Length of Hospital Stay**	*Median (IQR) Age*
Critical Care Group	45 (12.5)	23 (19)	8 (4.8)	2 (2)
No critical care group	315 (87.5)	96 (81)	3 (2.7)	1 (2.8)

*Table 9 Comparison between those who did and did not require critical care during hospital admission. *p<0.05*

3.2.4 Factors Affecting Second Visit to A&E

3.2.4.1 Clinical and demographic factors associated with the first admission

Overall, 221/479 infants re-attended hospital on at least one other occasion for a respiratory cause over the subsequent 12 months (*Multiple attendance group),* and 258 did not re-attend (*Single attendance group*) (Table 10). The median age at first attendance for both groups was 2 months (p=0.5). The median length of first hospital stay was significantly longer in those that subsequently re-attended (*Multiple attendance*, 4 (5) days; *Single attendance*, 3 (3) days; p=0.006). The proportion of females was lower amongst those in the multiple than in the single attendance group (Multiple 35%; Single 47%: p=0.006). The percentage of children who required nasogastric feeds or IV fluids during their first attendance was the same in both groups (64%). More children who subsequently re-attended hospital required critical care (*Multiple attendance* 19.9%; *Single attendance* 9.3%: p<0.05*).* The percentage of children who required oxygen during their first admission was similar in both groups (*Multiple* 70%; *Single* 69%*).* Differences in the relationship between viral group and hospital admittance were analysed using Chi-squared tests in the 221 that re-attended hospital. In the RV group 69 children re-attended hospital and 21/69 (30%) were admitted to hospital. In the RSV group, 152 infants re-attended hospital and 26/152 (17%) were admitted. This difference was statistically significant (p=0.025).

	Single Attendance Group (n=258)	Multiple Attendance Group (n=221)
Number of females*	122	77
Median (IQR) Length of Stay*(Days)	3 (3)	4 (5)
Median (IQR) Age (months)	2 (2)	2 (2)
Supportive feeds or fluids (number: %)	164 (64%)	142 (64%)
Critical care admission (number: %)*	24 (9.3%)	44 (19.9%)
Oxygen administration (number: %)	177 (69%)	155 (70%)
RSV Infection (number: %)	208 (58)	152 (42)
Rhinovirus Infection (number: %)	50 (42)	69 (58)

Table 10 Comparison between those who visited A&E once only and those who had multiple attendances.
**p<0.05*

3.2.4.2 Survival Analysis (time to second attendance) based on:

i. Virus detected at first visit

The proportion of infants infected with either RSV or RV during their first attendance who re-attended hospital for a respiratory cause, and the time taken to re-attend hospital/A&E was analysed using a Kaplan Meier plot (Figure 15). Overall, a higher proportion of those previously infected with RV (58%) than RSV (42%) re-attended hospital in the subsequent 12 months (p=0.003). Children previously with RV re-attended earlier than those with RSV (204 vs 267 days: p=0.001).

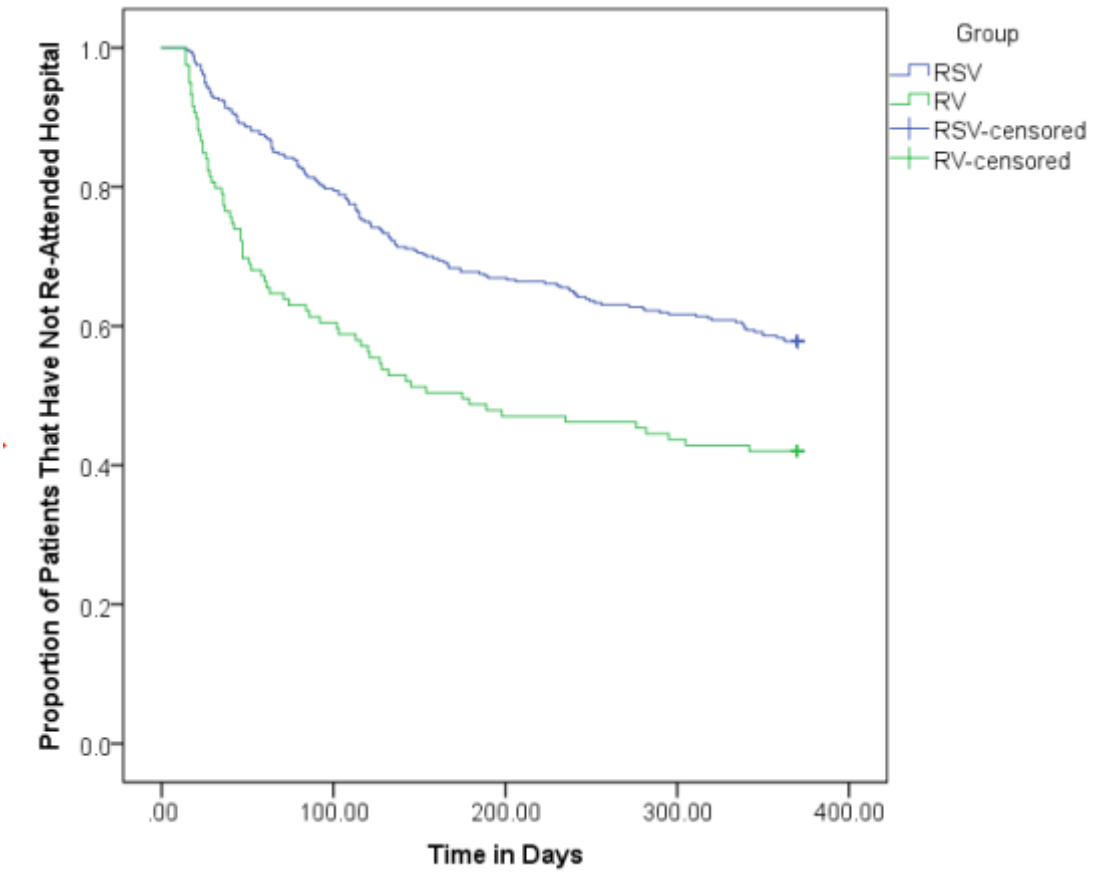

Figure 15 Kaplan Meier graph of the time taken to attend hospital for the second time based on viral group Log – Rank (Mantel-Cox) p=0.001

ii. Age at first visit

Age at first visit was dichotomised into those infants less than (n=329) or greater than (n=150) 3 months on their first attendance. Figure 16 shows that 144/329 (44%) of those $<$ 3 months re-attended hospital with a mean length of 260 days, and 77/150 (51%) of those $\geq$ 3 months re-attended hospital a mean of 233 days later. These differences were not significant; Log Rank (Mantel-Cox) p=0.10.

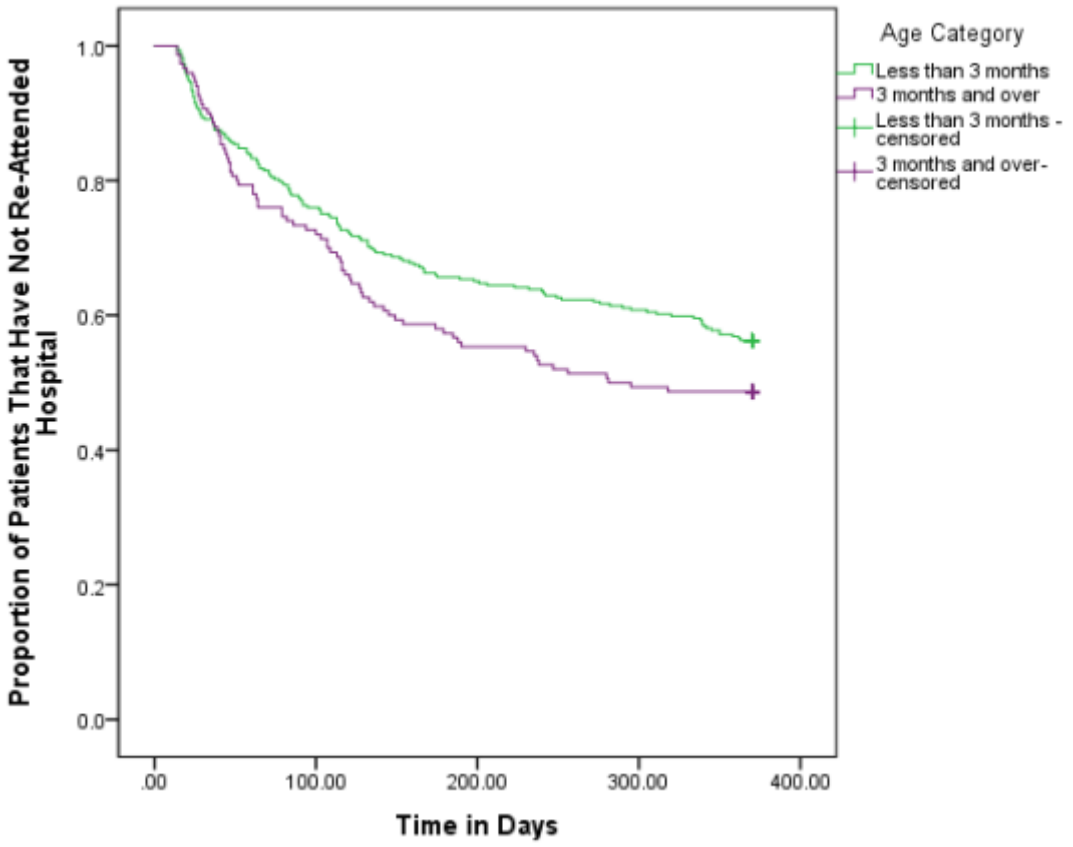

Figure 16 Kaplan Meier based on age on first presentation, Log Rank (Mantel-Cox) p=0.10

iii. Need for supplementary feeding/IV fluids during first visit

Data were dichotomised into infants that needed (n=306) and did not need (n=173) supplementary feeding or IV fluids during their first admission. Figure 17 shows that 142/306 (46%) of those infants who did require supplementary feeding or IV fluids during their first admission re-attended hospital a mean of 252 days later, and 79/173 (46%) of those that did not require supplementary feeding or IV fluids re-attended a mean of 251 days later. These differences were not significant; Log Rank (Mantel-Cox) p= 0.978.

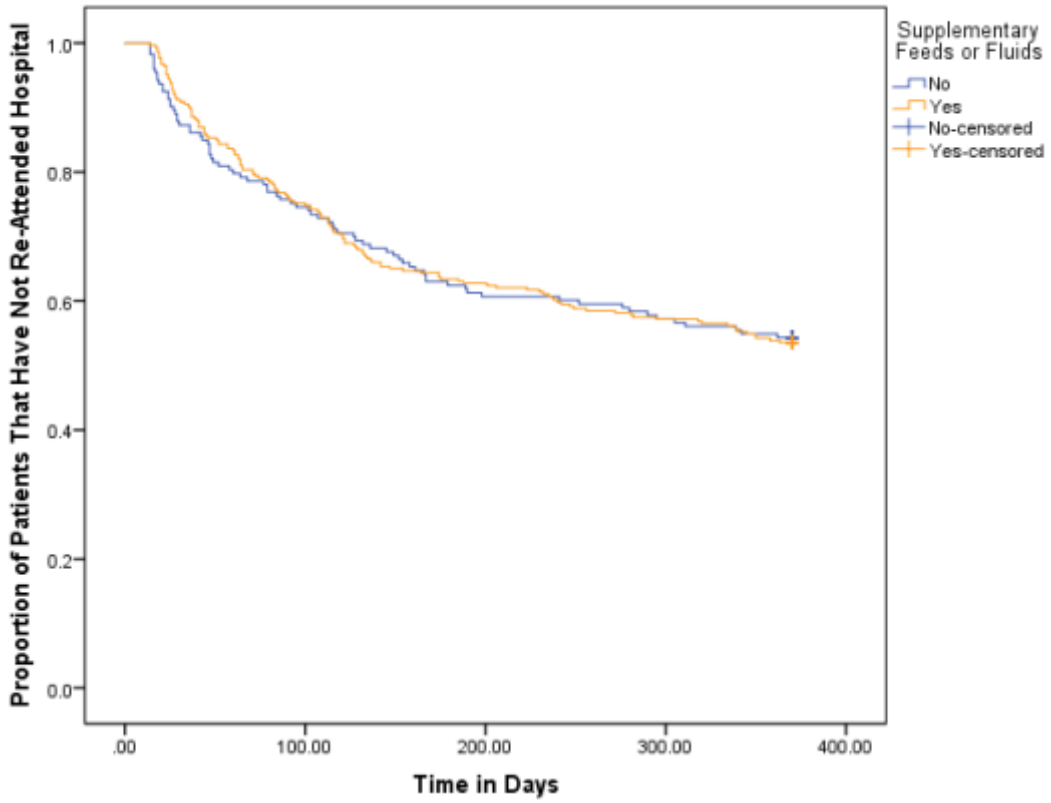

Figure 17 Kaplan Meier graph based on supplementary feeds or fluids, Log Rank (Mantel-Cox) p= 0.978

iv. Need for supplementary oxygen during first visit

Data were dichotomised into those infants that did (n=332) and did not need (n=147) supplementary oxygen during their first admission. Figure 18 shows that 155/332 (46%) of those infants who did require supplementary oxygen during their first admission re-attended hospital a mean of 248 days later, and that 66/147 (44%) of those that did not require oxygen re-attended a mean of 258 days later. These differences were not significant; Log Rank (Mantel-Cox) p = 0.652.

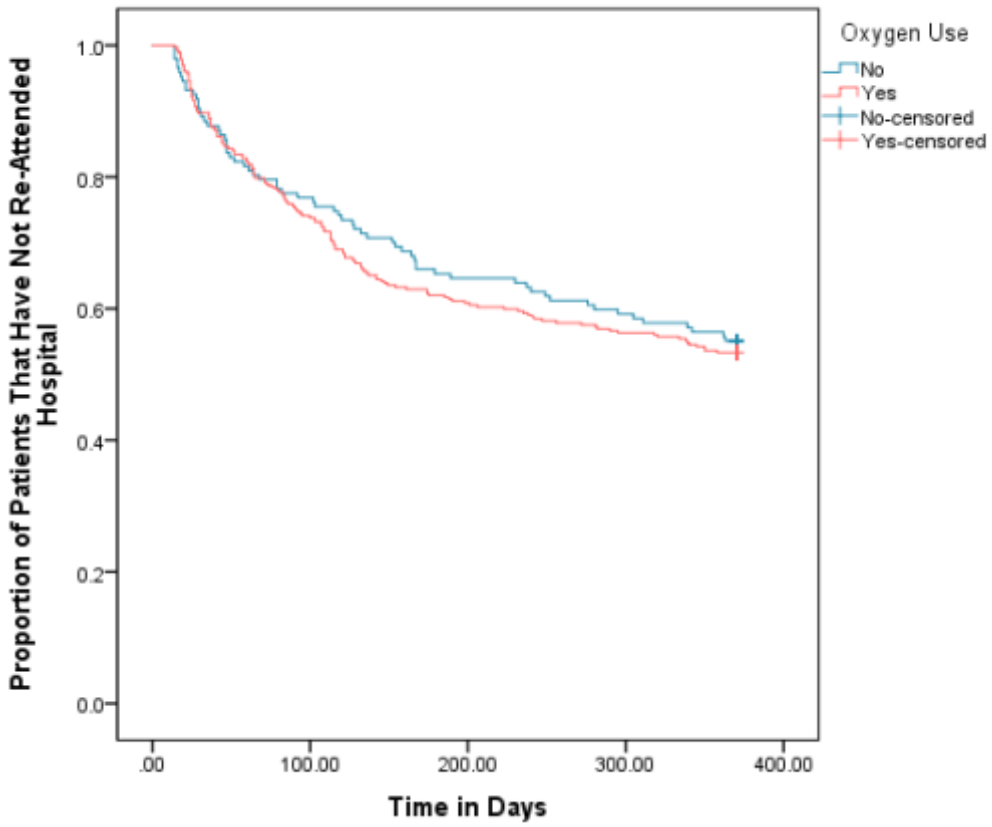

Figure 18 Kaplan Meier based on oxygen use during first visit, Log Rank (Mantel-Cox) p = 0.652

v. Need for critical care during first visit

Data were dichotomised into those infants that did need (n=68) and did not need (n=411) critical care during their first admission. Figure 19 shows that 44/68 (65%) of those infants who did require critical care during their first admission re-attended hospital a mean of 186 days later, and that 177/411 (43%) of those that did not require critical care re-attended a mean of 262 days later. These differences were significant; Log Rank (Mantel-Cox) p<0.01.

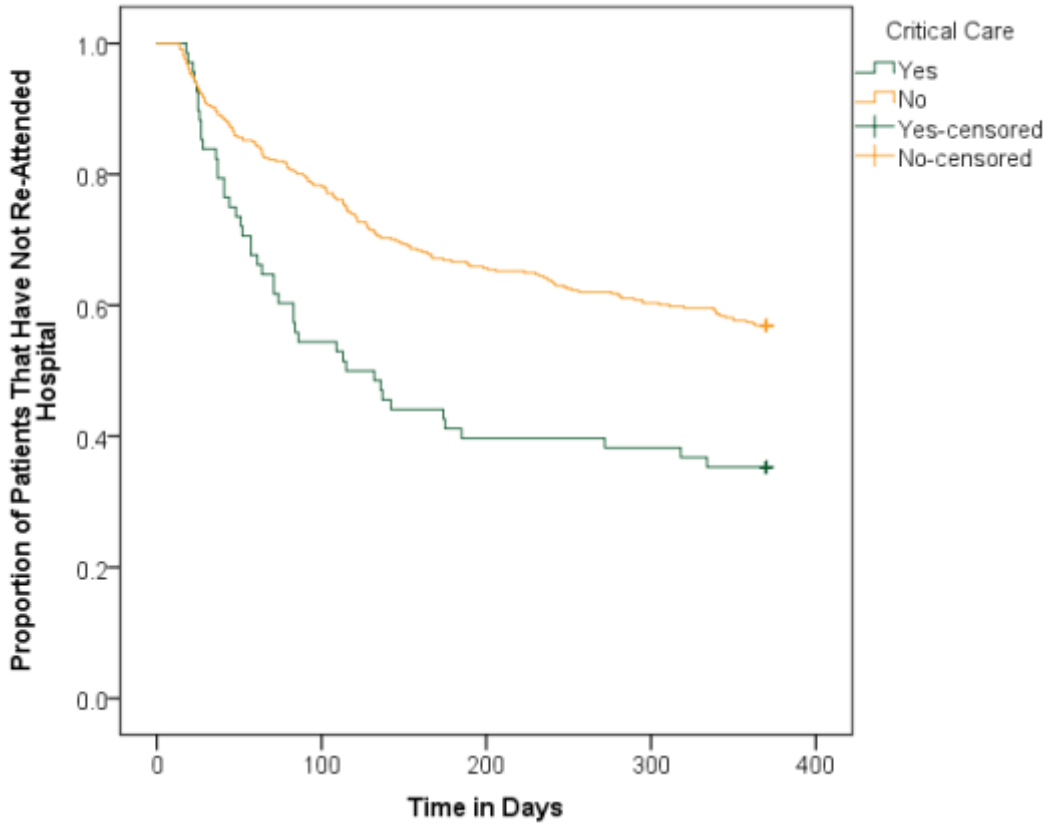

Figure 19 Kaplan Meier graph based on critical care admission, Log Rank (Mantel-Cox) p<0.01

vi. Cox Regression Survival analysis

A cox regression survival analysis was carried out to examine the effect of multiple variables on time taken to re-attend hospital. The two independent factors that affected risk of multiple attendances to hospital were viral group and a critical care stay during first visit. A hazard ratio (HR) of 1.6 (95%CI 1.2-2.2) was calculated for those in the RV group (p=0.001) compared to the RSV group. A hazard ratio of 1.9 (95% CI 1.3-2.7) was calculated for those who were admitted to critical care compared to those who did not (p=0.001). Oxygen requirement was not an independent risk factor for re-attending hospital, (hazard ratio 1.1; (95% CI 0.8-1.5) p=0.569). Being younger (≤ 3 months) and having had supplementary feeding/IV fluids were found to be protective (HR 0.8 (95% CI 0.6-1.0) and 0.9 (95% CI 0.7-1.2) respectively) but these relationships were not found to be significant.

Variables in the Equation

	B	SE	Wald	df	p value	Hazard Ratio	95% Confidence Interval	
							Lower Limit	Upper Limit
Viral Group	.493	.153	10.392	1	.001	1.637	1.213	2.208
Critical Care	.628	.187	11.287	1	.001	1.875	1.299	2.705
Three months	-.233	.142	2.703	1	.100	.792	0.600	1.046
Oxygen Use	.096	.169	.325	1	.569	1.101	0.790	1.534
NG Feeding	-.111	.162	.474	1	.491	.895	0.651	1.229

Table 11 Cox-Regression Model for multiple A&E attendances

3.2.5 Discussion

In this chapter, the clinical and demographic characteristics associated with RSV and RV infection in infants under six months who attended A&E was studied. The relationship between factors associated with first admission and time taken to re-attend hospital with respiratory symptoms were also studied. Distinct differences were found between those admitted to hospital with respiratory symptoms caused by RSV or RV infection. On first presentation, infants with RSV infection required more oxygen, nasogastric feeding and had a longer duration of hospitalisation than those admitted with a RV infection. Not surprisingly, infants admitted to critical care were more likely to re-attend hospital during the subsequent year. However, counter-intuitively, infants who had a RV infection, were more likely to re-attend hospital during the subsequent year than infants with RSV infection.

There are numerous studies that have looked at the effect and clinical characteristics of acute LRTIs in infancy. However, few were restricted to infants on their first hospitalised LRTI episode. A French multicentre study on 209 infants was carried out by Marguet *et al*. Investigators looked at the severity of illness through oxygen saturation on admission, length of oxygen supplementation, length of hospital stay and clinical severity score in infants on their first bronchiolitis episode. The median age of infants in this study was 2.4 months which is like our cohort. Marguet's paper compared those with a single RSV infection to those with RV, RSV/RV coinfection and human metapneumovirus (hMPV). Children that were RV positive were compared to RSV positive infants and had a decreased risk of requiring oxygen (OR 0.29, 95% CI 0.09–0.90) and hospitalised for more than 5 days (OR=0.11, 95% CI 0.03–0.37). Comparing the coinfection group to the single viral groups, revealed that a statistically significant higher proportion of the coinfection group required oxygen than the RV group p=0.05. In addition to this, the RSV group had a higher proportion of infants hospitalised for more than 5 days p=0.03 (128).

A Greek study (Papadopoulos *et al.)* also recruited healthy full-term children under 18 months with bronchiolitis provided they had not had more than two wheezing episodes in the past. The study looked at the differences in disease severity and patient characteristics based on viruses identified. On hospital presentation, infants' severity scores were calculated using a predetermined questionnaire and relevant medical history was collected. Papadopoulos found a statistically significant difference in ages between RSV, RV and RSV/RV coinfection group (RSV=3.2 months, RV=5.2 and RSV/RV=3.7 months) but this was not seen in our cohort. In Papadopoulos' cohort infants with RV were more likely to have a clinical score above the 50th decile, (OR: 4.9, 95% CI 1.2-18.7; p=0.022) compared to other groups,

which is contrary to our findings. Children with a coinfection were more likely to have a clinical score above the 50th percentile but this did not reach significance OR, 3.7 (95% CI, 0.95–14.5; p=0.059) (129).The differences in results could be explained by the smaller sample size used by Papadopoulos as only 87 infants had a virus detected which were then compared by virus group. Having such small groups for comparison affects the reliability of results.

Our study went on to look at the short-term respiratory outcomes of an early LRTI. The Kaplan-Meier plots showed obvious differences in time taken to re-attend hospital between those infants who did and did not require critical care, and between those with RSV and RV infection. These differences were confirmed using a cox-regression model. There are several reasons why a critical care admission might result in early re-attendance to hospital. Parents may experience increased anxiety after a serious admission and have a lower threshold for re-attending hospital with later respiratory symptoms. Perhaps more likely is that a serious LRTI, potentially resulting in the use of CPAP or invasive ventilation, might damage the lungs making them more susceptible to subsequent respiratory viral infections and therefore possible hospital attendance.

Given that a RSV infection resulted in more severe disease on first admission, it was surprising that RV infection was associated with earlier re-attendance. However, our findings are in line with several other studies. An Italian (Midulla *et al.)* cohort study recruited full term infants on their first episode of acute LRTI. A year after discharge parents were interviewed about their child's health and asked about recurrent wheeze, which was defined as two or more physician verified episodes of wheeze. Participants with acute LRTI were compared to a control group who were attending hospital for non-respiratory reasons. Infants in the LRTI group had a median age of 2 months like our cohort. A multivariate logistic regression model found that initial RV infection (OR 3.3, 95% CI 1.0-11.1; p<0.05), positive family history of asthma (OR 2.5, 95% CI 1.2-4.9; p<0.01) and absence of lung consolidation on chest x-ray (OR 2.6, 95% CI 1.1-6.1; p<0.04) were risk factors for subsequent recurrent wheeze (130). Looking specifically at RSV and RV, 43% versus 80% suffered from recurrent wheezing. This study and others like it suggest that the infective virus does play a role in the subsequent health of a child.

A Finnish cohort of 81 children recruited on their first hospital episode for a LRTI before their second birthday, were followed for 11 years (131). On initial presentation, the majority of children who were RSV positive were under six month (65%) whereas those with RV were

over six months (93%) (132). 58% of those who had rhinovirus infection when first hospitalised had a diagnosis of asthma at 11 years, compared to 20% of those who had RSV infection. For children who initially had a RSV infection, the crude odds ratio (OR) for asthma at 11 years was 0.269 (95% CI:088-0.816) compared to those infected with another virus who did not have asthma (p=0.016). However, after adjusting for confounders, significance was lost (OR: 0.407, 95% CI 0.114–1.450; p>0.05). For children initially with RV infection, the crude OR of having asthma at 11 years was 2.664 (95% CI 0.894–7.943; p<0.05). Similarly, after adjustment for confounders, these results also lost significance (OR:1.411, 95% CI:0.403–4.938; p>0.05) (131). These findings provide some evidence that significant RV infection in the early years of life may be associated with significant respiratory morbidity many years later but bigger studies will likely be needed to show this.

The COAST cohort study was the first large study to examine differences in outcomes between children at high risk of developing atopy/asthma who were infected with RSV and RV in early childhood. Infants with a positive atopic family history were recruited to help identify risk factors for an asthma diagnosis at 6 years. NPA samples were collected at clinic visits (at 2, 4, 6, 9, and 12 months of age). They were also collected during times of acute respiratory illness. Investigators were told by parents when a respiratory illness occurred, and then visited the family if a child scored more than 5/31 on a predetermined severity score. Children who wheezed with RV during infancy were at greater risk of having asthma at age 6 than children who did not wheeze with RV or RSV. This was true whether they wheezed only with RV (OR: 2.9; 95% CI: 1.1-7.5) or had additional wheezing illnesses with RSV (OR, 2.7; 95% CI, 1.2 - 6.3) during this same period. In contrast, children who wheezed only with RSV during infancy developed asthma at age 6 at a rate similar to those who did not wheeze with RV or RSV (OR, 1.2; 95% CI: 0.4-3.2). The presence of food or environmental allergies during infancy were also independent factors for asthma at 6 years (133). A limitation of our study is that we do not know how prevalent a personal or family history of atopy was in our cohort of patients. It is possible that this could have had a bigger impact on re-attendance (with or without RV infection) than just RV infection alone.

As mentioned in chapter 1, rhinovirus C (RV-C) is commonly found in respiratory secretions from young children presenting to hospital with viral induced wheeze/asthma exacerbations (52–54)**.** A prospective study by Cox *et al.* on ~200 pre-school children presenting to hospital with acute wheeze showed that RV was the most commonly identified virus and RV-C was the most common RV species. Overall, RV increased the risk of hospital re-admission after recruitment compared to those infected with another virus (RR: 3.44, 95% CI 1.17–10.17;

p=0.03). RV-C, was associated with an increased risk of a respiratory hospital admission before (49.4% vs. 27.3%, respectively; p = 0.004) and within 12 months (34.6% vs. 17.0%; p = 0.01) of recruitment. Risk for subsequent ARI admissions increased further in atopic subjects. This further highlights the importance of a RV-C as a possible marker of recurrent wheeze or asthma development (120).

A potential criticism of the Cox study was that participants were not recruited on their first hospital attendance for a respiratory symptom so any effects could have been due to previous infections. A more recent study looking at children on their first wheezing episode aimed to look at the short-term effect of infection with different species of RV on respiratory health (134). Children infected early with RV-A and RV-C were more likely to wheeze in the first 12 months than children with a non-RV infection. Children with RV-A or C were also more likely to suffer from subsequent RV-induced wheezy episodes. This cohort on recruitment was different to our study; the mean age was 12 months and the top three viruses detected were rhinovirus, bocavirus and RSV. However, our work and this study suggest that early rhinovirus infection, irrespective of species, is an important risk factor for recurrent wheezing in early childhood and may be an indicator of underlying susceptibility to recurrent wheeze/asthma (134).

3.2.5.1 Limitations

There are several limitations to our study. Firstly, this study only took place at one site and findings may not be generalizable to other centres either nationally or internationally because of differences in local practice e.g. routine viral testing is not done at all hospitals. Although 46% (221) of infants re-attended A&E for a respiratory reason, only 10% (47) of the original population were re-admitted. Parental perception may be a contributing factor to the high re-attendance rate. The general population may use Alder Hey A&E as a primary care resource as they have the ability to access the Emergency Department without referral, so parents may by-pass their GP meaning that attendances may not reflect a true need for hospital attendance. A hospital admission to Alder Hey may be perceived differently by parents. Alder Hey is also quaternary paediatric hospital so children will be transferred from other hospitals to Alder Hey for specialist care in severe cases. Due to this, parents may perceive mild/moderate illness as severe simply due to the fact they were admitted to Alder Hey hospital. This may increase guilt for not seeking medical help sooner or have anxiety post admission and consequently lower the threshold for seeking medical help and make them less likely to utilise their community healthcare services.

Lastly, and this is a problem with all such studies, we cannot be sure that RSV or RV caused the symptoms seen in these infants. Coinfection with multiple viruses and bacteria is common at this age, for example there was surprisingly high number of pertussis infection in children with RV infection although the RV group appeared to have less severe disease. Furthermore, common bacterial infections were not tested for in this group such as *S. pneumoniae*.

3.2.5.2 Strengths

This is a pragmatic retrospective observational study on a large cohort of infants under six months of age with respiratory illness caused by RSV or RV. It is only relatively recently that we have been able to analyse respiratory samples routinely for multiple pathogens. Five years ago, this service evaluation would not have been possible and it would likely not be possible currently in many other centres in the UK. We did not restrict to a specific disease on presentation which gives a more accurate picture of the effect of the two viruses.

3.2.5.3 Recommendations

To see the true effect of RSV and rhinovirus, primary care information from GPs and walk-in centre visits would be needed as well as a control group of children attending A&E for non-respiratory reasons. Looking at parental reasoning behind A&E attendance in conjunction with actual severity of symptoms, education levels, understanding of healthcare infrastructure/resources would also be of use. This may highlight areas of improvement in communication to parents when explaining intervention during hospitalisations and prognosis to help reduce parental anxiety and in turn hospital attendances. Furthermore, looking more closely at individuals' risk factors, such as maternal smoking during pregnancy, birth weight, passive exposure to cigarette smoke as well as genotype could highlight those who are more at risk of recurrent wheeze and their proportion in this cohort. Lastly, RSV and RV viral load could be measured to attempt to determine whether infection was causing symptoms or just due to carriage.

3.2.5.4 Conclusion

Our study shows that there are distinct differences in the outcomes of RSV and RV infection when occurring in children $\leq$ six months when causing hospitalisation for the first time. Although children admitted with RSV infection had more severe disease (more oxygen, longer length of stay), a higher proportion of those with rhinovirus infection re-attended A&E within the 12-month follow-up period. These differences fit well into published literature

and allude to rhinovirus infections being a possible predictor or cause of the development of asthma.

4. Discussion

In this thesis, several areas surrounding viral respiratory tract infections in pre-school children were explored. In the introduction, the disease burden of viral respiratory infections was highlighted and the growing need for a bronchiolitis severity score in both clinical practice and research. The association between an early lower respiratory tract infection, atopy, recurrent wheeze and asthma development was also identified.

Chapter 2 focussed on validating the Liverpool Infant Bronchiolitis Severity Score by assessing the responsiveness of the score to the Paediatric Early Warning System. Children were recruited and examined using the LIBSS scoring system. This project did not answer the original aim of the study due to a small sample size and short observation period, but provided useful information on how to improve protocol to fully validate work.

In chapter 3, the clinical and demographic differences between an early RSV and RV respiratory tract infection in children six months and under was investigated. The service user evaluation retrospectively compared the severity of initial viral infection based on intervention and length of stay during first hospital visit. Short-term respiratory morbidity was also explored by following patients up for one year to see if there were any A&E attendances for respiratory reasons. There were distinct differences in RSV and RV severity on initial presentation, infants with RSV had more medical intervention and a longer duration of stay, 4 days versus 3 days. RV and a critical care admission statistically increased the odds of re-attending A&E for respiratory reasons. Children with RV were 1.6 times more likely to re-attend A&E versus RSV. A critical admission increased the odds of re-attending A&E by 1.9.

4.2.1 Strengths

The work carried out in this thesis builds on basic clinical practices or knowledge already used in a clinical setting. The LIBSS is a short questionnaire with similar components to the PEWS already used in clinical practice so will be easy to implement in the future. We also aimed to use nasopharyngeal samples in future work to identify a biomarker as it is an established investigation in pre-school children with respiratory illness. The service user evaluation used information extracted from the hospital's electronic notes meaning any trends seen in treatment or attendance were not influenced by participant bias and can be carried out at

any point as no additional information was collected from patients. This means any potential findings from this work easy to implement into clinical practice.

4.2.2 Limitations

An overall limitation in this thesis is the lack of information about comorbidities and risk factors for respiratory illness in the children investigated. Parents in the LIBSS study were asked about risk factors for respiratory disease and paper and electronic notes were checked. For our service user evaluation, electronic notes were also reviewed for patient co-morbidities and family history. Although there was no other way to collect this information, there was limited information about co-morbidities as it relied on parent's remembering this information and healthcare team asking these questions and recording it. Some practitioners may not find information about parental smoking and history of allergies and atopy relevant when reviewing patients however this information would have been useful to our study as research has shown all these factors are somehow associated with asthma development (122,133).

4.2.3 Further Work

4.2.3.1 Liverpool Infant Bronchiolitis Severity Score

In future, it is possible that LIBSS may become a useful tool for assessing older children with bronchiolitis (particularly given that the recent NICE guidelines have extended the age at which children can be diagnosed as having bronchiolitis from 12 months to 24 months and possibly viral induced wheeze (12). There is also scope for the LIBSS to have prompts for intervention or investigation based on score like the PEWS. The ReSVinet score has also undergone vigorous validity testing, comparing these two scores would also be useful for potential users (39).

4.2.3.2 Service User Evaluation of Respiratory Related Attendances

The work carried out produced promising results that have opened up various study ideas. Observing similar trends in hospitals around the country could give further validity to findings and help guide hospital budgets. There could also be differences in trends of hospitalisation and A&E attendances between specialist paediatric hospitals and paediatric wards in general hospitals. Rhinovirus is an independent risk factor for re-attending hospital, sequencing these samples to find out whether RV-C is more prevalent in those who subsequently re-attended hospital would add considerably to our study. Continuing surveillance would also be beneficial to see if the strength of association diminishes over time.

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Appendices

Appendix 1: LIBSS Study Protocol

Study Team:

Chief Investigator: Professor Paul McNamara

Co-investigators: Dr. Brian Flanagan, Dr. Clare van Miert, Dr. Gemma Saint and Pearl Ampah

Statistician: Steven Lane

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CLINICAL QUERIES

Clinical queries should be directed to Pearl Ampah (hlpampah@liv.ac.uk) who will direct the query to the appropriate person.

FUNDER:

The student who is undertaking this research will be self funded. Laboratory consumables will be funded through existing grants and consultancy funds paid to Prof McNamara and held by university. A small amount of funding will also come from the Institute of Translational Medicine.

SPONSORSHIP:

The University of Liverpool is the research Sponsor for this Study. For further information regarding the sponsorship conditions, please contact:

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STUDY SUMMARY

Viral induced respiratory symptoms, particularly in the pre-school years, are a common cause of respiratory morbidity in children in the UK and worldwide. Bronchiolitis, an infection of the bronchioles (small airways in the chest) typically caused by respiratory syncytial virus (RSV), is one of the best described and commonest causes of respiratory symptoms in this age group. Until recently, the diagnosis of bronchiolitis was limited in the UK to children < one year of age but recent NICE guidance have increased this to 2 years in line with the USA and other parts of the world. Previously many children with 'bronchiolitis' symptoms caused by RSV between 1-2 years of age in this country were described as having viral induced wheeze.

Infection with viruses such as RSV in children under 2 years causes a spectrum of disease ranging from severe life-threatening bronchiolitis needing intensive care, to mild upper respiratory symptoms. However, overall 2-3% of all children are admitted to hospital with bronchiolitis in their first year of life. Once in hospital, treatment/management is mainly supportive, i.e. help with feeding and breathing but despite this, children can deteriorate very rapidly. As part of an NIHR-funded study, we have developed the Liverpool Bronchiolitis Severity score (LIBSS) to help healthcare professionals assess how sick a child is with bronchiolitis/viral induced wheeze.

The aim of this study is to see how well LIBSS works in children < 2 years of age admitted to hospital with bronchiolitis/viral induced wheeze and whether it could be used to predict those children that get worse. We also want to see whether LIBSS responses correlate with other markers of disease severity such as the amount of virus or levels of inflammatory proteins found in respiratory secretions in the nose.

GLOSSARY OF ABBREVIATIONS

ELISA Enzyme-linked Immunosorbent assay

hRSV Human Respiratory Syncytial Virus

IL-8 Interleukin 8

LIBSS Liverpool Infant Bronchiolitis Severity Score

NPA Nasopharyngeal fluid aspirate

PCR Polymerase Chain Reaction

PVB asthma Post-viral bronchiolitis asthma

ROC curve Receiver Operator Characteristic Curve

KEYWORDS:

Respiratory syncytial virus, viral induced wheeze, bronchiolitis, severity score, biomarkers, interlukin-8, neutrophil elastase, caspase, immune response

TITLE:

Longitudinal Validation of the Liverpool Infant Bronchiolitis Severity Score and Investigation into Predictive Severity Biomarkers in Children under Two Years with Bronchiolitis and Viral Induced Wheeze

DESIGN:

Quantitative interventional cross sectional study with questionnaires and human sample collection and analysis in healthy infants under 24 months.

AIMS:

To determine whether LIBSS responses with/without other markers of disease severity in children < 2 years of age admitted to hospital with bronchiolitis/viral induced wheeze can predict clinical deterioration.

OUTCOME MEASURES:

Longitudinal assessment of LIBSS responses and other markers of disease severity such as viral load and inflammatory cytokine concentrations in upper respiratory tract secretions

POPULATION ELIGIBILITY:

Healthy children between 0 to 24 months of age with a diagnosis of bronchiolitis or viral induced wheeze

DURATION:

20 months

Introduction

Background

Viral induced respiratory symptoms, particularly in the pre-school years, are a common cause of respiratory morbidity in children in the UK and worldwide. Bronchiolitis, an infection of the bronchioles (small airways in the chest) typically caused by respiratory syncytial virus (RSV), is one of the best-described and commonest causes of respiratory symptoms in this age group. Until recently, the diagnosis of bronchiolitis was limited in the UK to children < one year of age but recent NICE guidance have increased this to 2 years in line with the USA and other parts of the world. Previously, many children with 'bronchiolitis' symptoms caused by RSV between 1-2 years of age in this country were described as having viral induced wheeze.

Nearly all children under the age of two will experience a RSV infection however the severity varies.(1, 2) Most children will have coryzal symptoms whilst 1-3% of children will require hospitalisation for bronchiolitis. This spectrum of disease exists in children that do not have an underlying medical condition or known risk factors. Risk factors for bronchiolitis include:

Prematurity (<37 weeks)

Low birth weight

Babies between 0-4 months

Chronic lung disease or congenital defects of the airways (eg, cystic fibrosis, bronchopulmonary dysplasia)

Congenital heart disease

Neurological disease with hypotonia and pharyngeal problems

Immunocompromised children

Down's syndrome

Due to the variable nature of RSV infections clinical presentations and management differs.

In very extreme cases RSV infection can cause death, this is usually in immunocompromised infants or those with underlying health issues. For infants at high risk of severe RSV disease, palivizumab (a monoclonal antibody against the RSV F protein) is available as prophylaxis in order to prevent or reduce the severity of RSV infections. (3, 4)

Severity	**Mild**	**Moderate**	**Severe**
Symptoms	Mild fever	Same symptoms as a mild infection but to a greater degree	Same symptoms as a moderate disease but to a greater degree
	Cough	Wheeze and crackles on auscultation	Respiratory failure
	Increased respiratory rate and effort e.g. chest recession and nasal flaring	Dehydration >50% Apnoea	Pneumonia
	Runny nose	Low oxygen saturations	Cyanosis
	Difficulty feeding	Lethargy	Desaturations despite being on oxygen
Management	Increase fluid intake	Nasogastric feeding Oxygen	CPAP Intubation

Bronchiolitis, particularly when caused by RSV, has been linked to increased respiratory symptoms throughout childhood. Studies have shown there is a strong association between RSV infection and pre-school wheezing and/or asthma.(5) The type of asthma these children experience is oftentimes resistant to traditional asthma treatment and is referred to as “post-viral bronchiolitis” asthma (PVB asthma). The question is whether or not an early RSV infection causes so much damage to the lungs that a PVB asthma occurs. An alternative idea is that there is an underlying problem in certain children that makes them more susceptible

to severe RSV infection and asthma. The stimulation of the innate immune system by RSV could be a sign of natural disease clearing process or indicate a severe bronchiolitis infection(6). Looking at biomarkers and a severity score could identify if a rise or fall in various markers indicates an improvement or worsening of bronchiolitis.

Biomarkers including neutrophil elastase, interlukin-8, caspases and viral load will be investigated as part of this study in respiratory samples. As aforementioned, neutrophils are found in abundance within the airways of infants with bronchiolitis and viral induced wheeze. Neutrophil elastase is an enzyme released by neutrophils that causes the breakdown of proteins. If neutrophil elastase is released excessively, lung damage may occur. Elevated concentrations of neutrophil elastase could be an indication of lung inflammation in infants. Interlukin-8 (IL-8), is a cytokine known to attract neutrophils into the lungs during infections. It is important to look at IL-8 as the abundance of neutrophils could be due to the expression of IL-8.(7, 8) Caspase is also involved in the inflammatory process and programmed cell death (apoptosis). Caspase-1 has been shown to be increased in bronchiolitis and caspase 3 and 7 ratios alongside LDH have been investigated to see if they can predict severity of disease. (9, 10) Viral load from NPAs have been shown to reflect that of the lower respiratory tract. In addition, viral load has been linked to disease severity in infants without underlying medical conditions(11-15). Looking at trends in viral load, a known predictor of disease, alongside other biomarkers may highlight which biomarkers reflect a change in disease severity.

Rationale for Study

The Liverpool Infant Bronchiolitis Score has been partially validated by paediatricians and nurses according to FDA criteria. This included inter-rater, test re-test reliability testing and criterion testing but requires longitudinal validation, which we aim to address in this study. A clinical score would be extremely valuable for research and clinical practice. In research, the LIBSS would beneficial for quantifying illness severity and comparing the effectiveness of interventions. A score might also help standardize treatment, as there could be an agreed clinical picture and guidance of when to escalate and stop care. The combination of a severity score and an easily measured biomarker may enable prediction of disease course in order to guide hospital admission, treatment escalation and alert healthcare workers to the potential of deterioration in patients.

The Liverpool Infant Bronchiolitis Severity Score is an ideal clinical score for hospitals as it was developed with parents, nurses, healthcare assistants and doctors to ensure the most important symptoms and signs were included. The multidisciplinary approach to its

development means that all healthcare workers can use the score with confidence.(16) We have included both bronchiolitis and viral induced wheeze as it can be very difficult to differentiate between the two conditions (any distinction is likely artificial as symptoms/signs are similar and the same viruses often cause both conditions).(1, 2)

Figure 1 Distinguishing cause of cough associated with chest signs from NICE website (1)

	Pneumonia	Bronchiolitis	Viral-induced wheeze	Asthma
Age	Any age	Under 1 year, peak incidence at 6 months of age	First presents over 6 months, under 5 years	Over 12 months
Respiratory rate*	Usually increased	Usually increased	May be normal or increased	May be normal or increased
Hyperinflation	Not present	Often present (but difficult to detect in infants aged < 6 months)	May be present	May be present
Wheeze	Not usually present†	May be present	Present	Present
Crackles	Coarse crackles, usually localized	Fine crackles present throughout lung fields	Not present‡	Not present†

*May be associated with signs of increased work of breathing, subcostal, intercostal, and subclavicular recession. †Except with mycoplasma pneumonia. ‡ If present, usually clear with coughing.

Data from: [SIGN, 2006; Fleming and Elliot, 2007; Harris et al, 2011; SIGN and BTS, 2011]

Any predictive element discovered would need to have the potential to be implemented into clinical practice. This study will look for biomarkers in nasopharyngeal fluid aspirates (NPA) and nasal suction samples, as they are a part of standard diagnosis and supportive care in viral induced wheeze/bronchiolitis.(17, 18) Although there has been a lot work into biomarkers for disease severity,(19-21) plotting various biomarkers with LIBSS is more likely to yield a successful predictor as it takes into account the clinical picture of each participant.

Based on these aims there are two research questions generated:

Does the Liverpool Infant Bronchiolitis Severity Score reflect the severity of disease throughout the course a child's illness?

Can disease severity be predicted using LIBSS responses and other biomarkers of disease severity?

We hypothesize that the LIBSS will accurately reflect disease severity through the course of illness, and using the score in conjunction with biomarkers will predict severity of disease.

Study Objectives

Primary objective:

To complete validation of the LIBSS tool by using it longitudinally to assess disease severity in children with bronchiolitis/viral induced wheeze.

Secondary objective:

To assess whether LIBSS responses with/without other markers of disease severity can be used to predict clinical deterioration

Study Design

This quantitative study will combine results from the Liverpool Infant Bronchiolitis Severity Score (LIBSS) questionnaire with biomarkers from nasopharyngeal aspirates and or nasal suction samples to assess the severity of respiratory disease in hospitalised infants. The duration of study will be 20 months and we aim to recruit at least 40 infants between the ages of 0 – 24 months.

Twice daily, participants will be scored using the LIBSS, Monday to Friday. Most recent PEWS scores, ward location and impression from participant's direct health care team will be recorded whenever LIBSS is used. The LIBSS questionnaire will be carried out by the investigator or trained healthcare worker. Only those familiar with the LIBSS will assess patients to standardise data collected and consequently improve the precision of the results.

Nasopharyngeal aspirates are often taken on admission to hospital to determine which virus is causing the infection for cohorting reasons. We will 'scavenge' the remainder of this clinical sample. This has been agreed with James Cargill, consultant microbiologist at Alder Hey. In those patients in whom the parents agree, we will also collect NPA/nasal secretion samples from children on alternate days during patient's stay in hospital. Again, we will attempt to scavenge samples collected by nursing staff when suctioning the upper airways of these children. In those children who don't have routine suction performed we will seek permission from parents for this to be done. Samples will be analysed for viral load and inflammatory markers such as neutrophil elastase, caspase and interleukin-8.

Study Outcome Measures

The primary objective will be measuring correlation and agreeability between LIBSS severity of categories, viral load and biomarkers

The secondary objective will be measured by comparing the LIBSS score to direct health care team's opinion of severity of disease and PEWS score.

Participants

5.2.1.1 Target Population:

Infants under 24 months of age that have been diagnosed with viral induced wheeze or acute bronchiolitis according to NICE guidance.

5.2.1.2 Pre-Registration Evaluations:

General demographics and risk factors will be recorded for each patient at the start of the study. This will include any known medical conditions, medications, parental smoking, breast-feeding and older siblings with recent respiratory illness. A PCR is usually done as part of routine investigations in under twos with respiratory symptoms such as wheeze and crackles, if this is not done one nasopharyngeal sample will be taken by investigators.

5.2.1.3 Inclusion criteria:

Infants under 24 months with viral induced wheeze or acute bronchiolitis provided that parents/guardians understand English and have capacity to consent for their child.

5.2.1.4 Exclusion criteria:

Children with parents/guardians cannot consent to study due to communication barriers

Prematurity (infants that were born < 37 weeks gestation)

Those who have been given palivizumab

Immunocompromised patients

Severe underlying cardiorespiratory disease e.g. cyanotic heart disease, CF, severe tracheomalacia and bronchopulmonary dysplasia

Known neuromuscular disorder

5.2.1.5 Withdrawal Criteria

For families wishing to withdraw from the study their written information will be disposed of using confidential waste bins, and clinical samples disposed of in the appropriate way.

5.2.1.6 Adverse Events

Cross contamination and infection: Ayliff technique for hand washing, apron, face mask, and goggles will be worn by those collecting sample (as per Alder Hey guidelines). Specimens will be taken in child's bed area to prevent infection spread

Vomiting and possible aspiration: samples will be taken before meals or at least one hour after feeding.

5.2.1.7 Assessment and Follow Up

There will be no follow up for participants. The end of the study for individual participants will be when their direct healthcare deems their respiratory illness is adequately managed and discharges them from their care. Investigator will stop recruiting new participants in March (end of bronchiolitis season).

5.2.1.8 Statistics and Data Analysis

We aim to assess at least 40 participants. This is roughly around 12% of those who are identified using PCR for RSV bronchiolitis during bronchiolitis season (November-March) at Alder Hey. This number would account for an even smaller proportion of eligible patients if those with viral induced wheeze were included. There is no pilot data to use to establish the ideal number of recruits needed. However, when the LIBBS score was being developed target number was calculated as 12.5% of target population. Previous studies from our group have measured expression of cytokines and cell numbers from bronchoalveolar lavage, a fluid similar to NPA and numbers used vary between 47 and 182 recruits. A target number of 40 seems appropriate and feasible based on time and man power limitations. Due to the seasonal nature of bronchiolitis this study will happen over 20 months to ensure that 40 participants are recruited with the full amount of data. Participant's samples will be analyzed for presence and quantity of various biomarkers using laboratory tests (ELISA/multiplex bead arrays). Then the sample's code will be matched to corresponding LIBSS. Due to the small

sample size statistical analysis will be decided on once all data has been collected and basic statistical tests have been done e.g. mean, median of viral load and biomarker quantity for each severity category. Once all the data has been collected advice for the appropriate tests will be sought from a medical statistician. Based on sample size probable tests will be Mann-Whitney U tests, Pearson's correlation co-efficient and □2 test. At the end of the overall study samples and will be destroyed in accordance with University of Liverpool laboratories and the Human Tissue Authority's regulations.

Regulatory Issues

5.2.1.9 Ethical Approval

Ethical approval will be sought from NHS Ethics Approval via Health Research Authority Research Ethics Committee once successful sponsorship has been obtained from the University of Liverpool. The study will not commence until approval has been gained from NHS for the study and the participating site.

5.2.1.10 Consent

Participants will be identified through their healthcare team. Accident and emergency, general paediatrics and respiratory departments will be made aware of the study. Healthcare teams will identify any families that fit the criteria and approach them about the study. If families agree, the research team will then introduce themselves to families to explain the study and gain consent. Parents/carers will go through the aims, purpose and what the research involves with investigator, then written consent will be obtained. Investigators not involved in participant's care will not have access to identifiable data until consent to contact is gained. Three copies of a participant's consent form will be made: one will be given to families, another will be left in their patient notes to make members of their care team aware of their participation in the study and the third will be kept by investigators. For families that wish to be sent a brief abstract of our summary findings we will use child's Alder Hey number to get their address at the end of the study (provided consent is given).

5.2.1.11 Confidentiality

The Chief Investigator will preserve confidentiality of participants taking part in the study and will abide by the Data Protection Act. Any questionnaires from participants will be stored in a locked office at the Institute of Child Health, Alder Hey Children's NHS Foundation Trust. Any physical copies with identifiable information will be disposed of using confidential waste bins once they are no longer needed. Digital identifiable information will be kept on NHS password protected computers only. All data will be anonymised by assigning each

participant a unique randomised code for samples and questionnaires. Anonymised digital data will be password protected and stored on a secure computer network at either the Institute of Child Health at the University of Liverpool and or Institute in the Park (Alder Hey Children's NHS Foundation Trust).

5.2.1.12 Indemnity

Indemnity will be covered by MDU provided that study has received sponsorship/approval from the University of Liverpool and the NHS.

5.2.1.13 Sponsorship

Applying for sponsorship from the University of Liverpool.

5.2.1.14 Funding

Participants will not receive any incentives for participating in this study. The student who is undertaking this research will be self funded. Laboratory consumables will be funded through existing grants and consultancy funds paid to Prof McNamara and held by university. A small amount of funding will also come from the Institute of Translational Medicine.

5.2.1.15 Audits and Inspections

Investigators understand that the study may be subject to inspection and audit by the University of Liverpool under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

5.2.1.16 Study Management

The day to day management of the study will be coordinated by Pearl Ampah under the supervision of Prof. Paul McNamara, Dr. Brian Flanagan, Dr Clare van Miert and Dr. Gemma Saint.

5.2.1.17 End of Study

The end of the study for each participant will be when they are discharged from hospital or when their direct care team believes that they have recovered from their bronchiolitis infection (participants in hospital long term for another reason). Investigators will stop recruiting participants at the end of March 2017. Data will then be analysed. If recruitment target has not been met or data suggests higher numbers needed, further recruitment would take place during 2017-2018 season with the end of the study being July 2018. Samples will be destroyed at the end of the study in accordance with University of Liverpool laboratories and Human Tissue Authority’s regulations.

5.2.1.18 Archiving

Data will be archived for 15 years in the University of Liverpool repository

5.2.1.19 Dissemination of findings

The results of the study will be disseminated at a topic relevant conference, for example European Respiratory Society (ERS) Annual conference and published in a peer-reviewed journal. A brief precis of the study findings will be generated after all data has been analysed. Parents will be asked whether they would like to be sent this information at the end of the study, all information will be anonymized, and we anticipate it will be available from August 2018.

References:

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12. El Saleeby CM, Bush AJ, Harrison LM, Aitken JA, DeVincenzo JP. Respiratory syncytial virus load, viral dynamics, and disease severity in previously healthy naturally infected children. Journal of Infectious Diseases. 2011;204(7):996-1002.

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Appendix 2: Liverpool Infant Bronchiolitis Severity Score Template

Liverpool Infant Bronchiolitis Severity Score-Proxy Reported Outcome: Infant aged under three months			
Day of illness:			
1. Do you have any concerns relating to the infant's overall condition?			
No concerns (condition is stable or improving)	0		Comments:
Some concerns (may become unstable/requires close observation)	4		
Extremely concerned (unstable requires immediate medical review)	8		
2. Apnoea			
None	0		Comments:
Occasional self-correcting apnoea / short pauses	2		
Apnoea's increasing frequency & duration	4		
Apnoea's requiring stimulation	6		
Apnoea's requiring bag & mask ventilation	8		
3. Increased work of breathing (Absent or Mild =0) Please complete all boxes			
Moderate/severe recession	0	2	Comments:
Moderate/severe tracheal tug	0	2	
Moderate/severe nasal flare	0	2	
Moderate/severe head bobbing	0	4	
Grunting	0	4	
Central cyanosis (blue lips / tongue)	0	6	
4. % oxygen to maintain saturations ≥92% (or usual saturation level if infant has congenital heart defect)			
21% (room air)	0		Comments:
22 - 40% (0.02 - 6L/min)	2		
41 - 50% (7 - 10L/min)	4		
>50% (>10L/min)	6		
Actual amount of oxygen administered			
Mode of oxygen delivery: Nasal specs (NS); Face Mask (FM); Head box (HB); HiFlow (HF); nCPAP (CP)			
5. Respiratory rate (breaths per minute)			
25 - 59	0		Comments:
60 - 70	2		
<25 or >70	4		
6. Heart rate (beats per minute)			
105 - 165	0		Comments:
166 - 180	2		
<105 or >180	4		
7. Appearance			
Alert & active / normal sleep	0		Comments:
Irritable / fractious / restless	2		
Floppy / lethargic / poor interaction	4		
Only responds to pain / unresponsive	6		
AVPU			
8. Feeding			
>75% or normal amount of feeds via usual route	0		Comments:
50 - 75% of feeds of normal feeds via usual route	2		
<50% of feeds or needing NG feeds / IV fluids	4		
9. Urine output			
Usual number of wet nappies (> 2 mLs/kg/hr)	0		Comments:
Reduction in number of wet nappies (1 - 2 mLs/kg/hr)	2		
Small volumes of concentrated urine / no urine (< 1mL/kg/hr)	4		
10. Central capillary refill time (preferably press on the sternum for 5 seconds)			
≤ 2 seconds	0		Comments:
> 2 seconds	2		
Actual capillary refill time in seconds			

LIBSS Score Total: **Mild (0-10); Moderate (11-20); Severe (≥ 21)**		Comments:

Appendix 3: Consent form

Alder Hey Children's Hospital, Eaton Road,
Liverpool L12 2AP
Telephone: 0151 228 4811

IRAS ID: 215393
Participant Identification Number for this study:

CONSENT FORM FOR RESEARCH
For parent/person with parental responsibility

Title of Project: **Longitudinal Validation of the Liverpool Infant Bronchiolitis Severity Score and Investigation into Predictive Severity Biomarkers in Children Under Two Years with Bronchiolitis and Viral Induced Wheeze**

Name of Researcher: _____ Job title: _____

Please initial box

1. I confirm that I have read and understood the information sheet (version 5 date: 23/01/17) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my child's participation is voluntary and that I am free to withdraw my child at any time, without giving any reason. If I do withdraw, his/her medical care and legal rights will not be affected in any way. ☐

3. I understand that relevant sections of any of my child's medical notes or data collected during the study may be looked at by Pearl Ampah, Professor McNamara, Dr. Clare van Miert or Dr. Gemma Saint (investigators). I give permission for these individuals to have access to my child's medical records and study data. ☐

4. I agree for my child to participate in this study i.e. for my child to have two extra examinations twice daily and for the use of the remains of any respiratory samples ('snot') collected as part of routine care of my child. ☐

5. I agree for extra respiratory samples to be taken from my child on alternate days throughout my child's admission to hospital. ☐

6. I agree to anonymised data about my child such as age, gender and medical condition being held on a secure on-line database with limited access ☐

7. I agree for my child to take part in the above study. ☐

8. I agree to a member of the research team accessing my child's medical notes at the end of the study in order to local a postal address to send out a summary of the study findings. I understand that this will most likely be around August 2018. ☐

Name of patient _____

_____	_____	_____
Name of Parent/Guardian	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Appendix 4: Participant Information Sheet

Longitudinal Validation of Liverpool Bronchiolitis Severity Score and Investigation into Predictive Severity Biomarkers in Children Under Two Years with Bronchiolitis and Viral Induced Wheeze

IRAS ID: 215393

Participant Information Sheet

You and your child are being invited to take part in a research study. Before you decide whether you wish to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information.

What is this study about?

Bronchiolitis and viral-induced wheeze are common clinical manifestations of viral infections in children under-two. Symptoms include mild fever, runny nose, cough, labored breathing, and difficulty feeding. Although most of the time children with these conditions can be managed at home, 2-3% of all children need to come into hospital. Some just need extra fluids and oxygen but a small proportion need help with their breathing on intensive care. As symptoms can change very quickly, it can be very difficult to know which children need to come into hospital and what sort of treatment they need when they get there.

We have developed a severity score and want to see if the score, possibly combined with markers of infection found in your child's nasal secretions, can predict how severe their infection is and will be.

What will happen in the study?

Investigators will ask you general questions about your child and their health at the start of the study. E.g. is your child breast or bottle fed, how much did they weigh at birth and do you have any other children living at home.

Your child will be examined twice a day and responses to the Liverpool infant bronchiolitis severity score recorded.

- The examination will include taking your child's heart rate and breathing rate. Observing your child breathing and asking about their feeding and general well-being. This only takes 5 minutes to do.

It is likely that when your child came into hospital they had a 'snot' sample collected from their nose to find out what virus was causing their symptoms. What's left of this sample is stored in the laboratory and we would like to use the remainder to find out how much virus and various inflammatory proteins are the nose. Your child may also have 'snot' sucked out of their nose to help them to breath. We would like to save these samples and analyze them in the same way.

If your child hasn't had 'snot' samples sucked out of their nose on a regular basis, we would like to do this every other day during their admission to hospital. All samples will be destroyed at the end of the study.

Who can participate in this study?

Children under two years that have been diagnosed with bronchiolitis or viral induced wheeze

Advantages, Disadvantages and Risks

There will be no direct benefits to taking part in this study. The only advantages might be that your participation might help improve the care of future children with bronchiolitis and viral induced wheeze.

The disadvantages to this study that it will take up some of your and child's time. It can sometimes be uncomfortable for your child having snot sucked from their nose.

Do I have to take part?

No. It is up to you to decide whether you to take part in the study or not. You are free to withdraw from the research at any time and without giving a reason. Your decisions about this will not affect the standard of care your child receives.

If you would like to take part, you will be given this information sheet to keep and be asked to sign a consent form.

Will the information on my child be kept confidential?

Yes, all personal information will be kept confidential and secure. Only people involved in the study will have access to the information. This study will be published in a medical journal and part of student's thesis, but it won't be possible to identify individual children or parents from what is written. This study has been approved by the Liverpool Children's Research Ethics Committee, which includes lay members representing the local community.

A Summary of findings will be available at the end of the study. If you wish to receive a copy of this please initial the relevant box on the consent form to confirm that you are happy for a member of the research team to access you child's medical records at the end of the study in order to locate a postal address for this purpose. The summary of findings is likely to be available around August 2018.

Who will carry out this study?

This study will be carried out by Pearl Ampah a postgraduate research student, under the supervision of Professor P. McNamara, Dr. G. Saint, Dr. Clare van Miert and Dr. B. Flanagan.

What if you have any problems or would like further information about the study?

You can either contact:

Prof Paul McNamara
Institute of Child Health,
Alder Hey Children's Hospital,
Eaton Rd,
Liverpool, L12 2AP
email: mcnamp@liverpool.ac.uk

The Patient Advisory Liaison Service (PALS)
Alder Hey Children's Hospital
Eaton Rd
Liverpool, L12 2AP
Tele: 0151 252 5161
Email:pals@alderhey.nhs.uk

Appendix 5: Sponsorship Letter

Professor McNamara
Institute of Translational Medicine
University of Liverpool
Crown Street,
Liverpool
L69 3BX

LIVERPOOL HEALTH PARTNERS

Mr Alex Astor
Head of Liverpool Joint Research Office

University of Liverpool
Research Support Office
2nd Floor Block D Waterhouse Building
3 Brownlow Street
Liverpool
L69 3GL

Tel: 0151 794 8739
Email: sponsor@liv.ac.uk

06 December 2016

Sponsor Ref: UoL001266

Re: Sponsorship Approval

"Longitudinal Validation of Liverpool Infant Bronchiolitis Severity Score and Investigation into Predicative Severity Biomarkers for Respiratory Syncytial Virus Infection"

Dear Professor McNamara

After consideration at the JRO Non Interventional Sponsorship Sub Committee on I am pleased to confirm that the University of Liverpool is prepared to act as Sponsor under the Department of Health's Research Governance Framework for Health and Social Care 2nd Edition (2005) for the above study.

The following documents have been received by the Joint Research Office

Document title	Version	Date
Consent Form LIBSS	4	25.11.2016
Participant Sheet	4	25.11.2016
LIBSS NPA Protocol	4	25.11.2016

Please note this letter does NOT allow you to commence recruitment to your study.
A notification of Sponsor Permission to Proceed will be issued when governance and regulatory requirements have been met. Please see Appendix 1 to this letter for a list of the documents required.

If you have not already applied for regulatory approvals through IRAS you may now do so at https://www.myresearchproject.org.uk/Home.aspx.

In order to meet the requirements of the Research Governance Framework 2nd Ed 2005, the University requires you to agree to the following Chief Investigator responsibilities:

Appendix 6: HRA Approval Letter

NHS
Health Research Authority

Miss Pearl Ampah
36 Prince Alfred Road
Liverpool
Merseyside
L15 5BG

Email: hra.approval@nhs.net

21 February 2017
Amended and reissued 06 March 2017

Dear Miss Ampah

Letter of HRA Approval

Study title:	**Longitudinal validation of Liverpool Bronchiolitis Severity Score and investigation into predictive severity biomarkers in children under two years with bronchiolitis and viral induced wheeze**
IRAS project ID:	**215393**
Protocol number:	**UoL001266**
REC reference:	**16/LO/2275**
Sponsor	**University of Liverpool**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

IRAS project ID	215393

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net. Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **215393**. Please quote this on all correspondence.

Yours sincerely

Thomas Fairman
HRA Assessor

Email: hra.approval@nhs.net

Copy to: *Mr Alex Astor, University of Liverpool, (Sponsor Contact)*
Miss Lucy Cooper, Alder Hey Children's NHS Foundation Trust, (Lead NHS R&D Contact)
Professor Paul McNamara, (Chief Investigator and Academic Supervisor)

Appendix 7: Ethical Approval Letter

NHS
Health Research Authority

London - South East Research Ethics Committee
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 104 8006
Fax:

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

30 December 2016

Miss Pearl Ampah
36 Prince Alfred Road
Liverpool
Merseyside
L15 5BG

Dear Miss Ampah

Study title:	**Longitudinal validation of Liverpool Bronchiolitis Severity Score and investigation into predictive severity biomarkers in children under two years with bronchiolitis and viral induced wheeze**
REC reference:	**16/LO/2275**
Protocol number:	**UoL001266**
IRAS project ID:	**215393**

The Proportionate Review Sub-committee of the London - South East Research Ethics Committee reviewed the above application on 21 December 2016.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Mrs Margaret Hutchinson, nrescommittee.london-southeast@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
IRAS Application Form [IRAS_Form_15122016]		15 December 2016
IRAS Checklist XML [Checklist_15122016]		15 December 2016
Other [Confirmation of procedures]		20 December 2016
Participant consent form [LIBSS VIW CF]	4.0	25 November 2016
Participant information sheet (PIS) [PIS LIBSS VIW]	4.0	25 November 2016
Research protocol or project proposal [LIBSS NPA v4]	4.0	25 November 2016
Summary CV for Chief Investigator (CI) [Dr Paul Mcnamara]		18 December 2015
Summary CV for student [Pearl Ampah]	1	01 November 2016
Summary CV for supervisor (student research) [Gemma L. Saint]		
Summary CV for supervisor (student research) [Dr Clare van Miert]		18 January 2016
Summary CV for supervisor (student research) [Dr Brian Flannagan]		
Validated questionnaire [LIBSS v10]	10	17 June 2015

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and

Appendix 8: Audit Registration

Alder Hey Children's NHS
NHS Foundation Trust

28 September 2016

Direct Line: 0151 252 5189
Email: sarah.stephenson@alderhey.nhs.uk

Quality and Governance Team
Clinical Audit
Interim Site
Building 1

Ms Pearl Ampah
Medical Student
Respiratory
Medical Specialties
Alder Hey

Dear Pearl,
Audit reference number: **5343**

Audit title: **The impact of different respiratory virus infections in infants on the subsequent prevalence of respiratory symptoms in pre-school children.**
Audit level: **Local audit project (data to be collected and retained at Alder Hey)**

Thank you for registering the audit named above with the Clinical Audit Department. This audit has been entered onto our database, and has been assigned a unique reference number **(5343)**.

I would appreciate it if you could please keep a note of this number, as in order to deal with queries and requests for support efficiently and effectively, you will be asked to provide this number during any future contact with the Clinical Audit Department. You may also find that this number is required by the Medical Records Department and/or the Information Department in order to access data for your audit.

The Clinical Audit Department is required to report regularly to the Trust and CCG's on the progress of all audits. I would therefore be grateful if, on completion of your audit, you could provide me with a copy of your audit report, complete with conclusions and recommendations. Wherever possible, recommendations should be time scaled and a 'person responsible' identified for taking forward each recommendation. Similarly, if this audit does not proceed for any reason, please let us know. If we do not hear from you, a member of the Clinical Audit team will contact you for this information.

You registered this audit as: "Local audit" you have specified: "No additional support required", in the support section.

Please contact Steve on ext 2636, Julie on ext 2562, Liz on ext 2409, Mary on ext 2856 or myself on ext 2189, if the level of support you specified changes. We will try our best to help you.

Further information about the Clinical Audit Department can be found on our intranet page under 'Clinical Audit'.
Yours sincerely

S. Stephenson

Sarah Stephenson
Clinical Audit & Compliance Manager