Acute infant bronchiolitis; management and prognosis

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Postbronchiolitis symptoms

In bronchiolitis we must now contend
with both the disease and the "now" and the then";
For many such infants a mold has been cast,
perhaps by their unborn and unknown past,
which destines that they shall in time wheeze again.
For them this disease
is the distant, boding knell
Of vulnerable lungs
to a microbe's mystic spell.

Hall CB, McBride JT. Bronchiolitis: In*: Mandell GL, Bennett JE, Dolin R, editors. Principles and Practice of Infectious Diseases. 5th Edn. Philadelphia, Churchill Livingstone, 2000; pp. 710—717*

To Marita, Andrea & Martine

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1 Preface

1.1 Acknowledgements

Being part of the Oslo Research group of Asthma and Allergy in Children, the Lung and Environment (ORAACLE) and the study team of the Bronchiolitis ALL study has been encouraging and important for my medical career and my education in paediatric lung and allergic diseases. Throughout the past decade, my research in bronchiolitis has followed my specialization in paediatric diseases, as a part-time physician and part-time PhD- student.

These studies, as a part of the Bronchiolitis ALL study, were only made possible because of the children and their parents' willingness to participate in the study. I am truly grateful for their effort during the hospitalisation and for their participation in the follow-up study. I am also overwhelmed by the effort of the local principal investigators and hospital staff of all the 8 participating hospitals; Innlandet Hospital Trust; Elverum and Lillehammer, Vestre Viken Hospital Trust; Drammen, Vestfold Hospital Trust, Telemark Hospital Trust, Sørlandet Hospital Trust; Kristansand, Oslo University Hospital Trust and Østfold Hospital Trust. You did an outstanding job of including infants with acute bronchiolitis and providing them with thorough follow-up during hospitalisation according to the study protocol.

My research project would never been carried out without the financial support of the research department at Østfold Hospital Trust and Oslo University Hospital during my period of employment up until October 2015.

This project would also have been impossible without the continuous support and encouragement from my supervisors, colleagues, friends and my dearest family. My gratitude is indescribable.

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I also want to thank The Bronchiolitis ALL study research group, in particular **Leif Bjarte Rolfsjord**, **Teresa Løvold Berents**, **Karin Eline Stensby Bains** and **Live Nordhagen**. Our great team effort has paid off and changed national and international guidelines in bronchiolitis management.

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For the past 4 years I have been working part-time as a consulting paediatrician in lung and allergic diseases at Department of Paediatrics and Adolescent Medicine at Østfold Hospital Trust. I am grateful for this opportunity and for the time provided to me to finish my PhD. Your patience and support are highly appreciated.

Finally, I would like to thank my great family: **Astrid** and **Odd Helge**, my parents, for their love, continuous support and always reminding me of what is important in life, **Eli- Karin** and **Gunnar**, my parents in law and my brothers; **Helge** and **Knut** and their families for their love and great support and my grandfather; **Olav**, sharing his wisdom with me and helping me to see life in a bigger perspective.

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Fredrikstad, May 2020

Jon Olav Gjengstø Hunderi

1.2 Summary of the thesis

Introduction

Acute bronchiolitis is a viral lower respiratory tract infection, mostly predominant during winter seasons and is commonly caused by respiratory syncytial virus (RSV), while other viruses may also be involved. Upper respiratory tract symptoms are most common the first days after incubation followed by lower respiratory tract symptoms such as cough, tachypnoea, wheezing and chest retractions. The course of the bronchiolitis varies from mild symptoms with no respiratory distress to severe disease with the need for non-invasive and sometimes invasive respiratory support. Children with mild bronchiolitis can be managed at home. Still, it is a major cause of hospitalisation among infants and children in their first year of life. How to best manage these children in the emergency ward and after hospital admission has been an object of study for decades. Few treatment options are found to reduce severity or shorten the duration of symptoms. The best hospital management is supportive, administrated when feeding difficulties, oxygen desaturation or respiratory distress occur. Sometimes, infants and children are hospitalised due to risk of rapid deterioration.

Prior to 2013, inhaled racemic adrenaline was the drug of choice in the initial treatment of acute bronchiolitis in Norway. Some studies have described short-term symptomatic effects from inhaled racemic adrenalin, but the effect on length of hospital stay (LOS), disease severity and use of supportive care have been found to a lesser extent. The Inhalations have mostly been given at fixed schedules but sometimes on demand. Studies comparing the efficacy of different inhalation strategies were lacking.

Several scoring tools to assess severity and effect of treatment of acute bronchiolitis have been developed combining objective and subjective parameters. However, their validity, reliability and clinical utility are questioned, and few previous studies have assessed parental ability to evaluate disease severity of their child during acute bronchiolitis.

Acute Bronchiolitis in infancy is known to increase the risk of later asthma development, as is allergic sensitisation. Studies have suggested a synergistic role of viral lower respiratory tract infections, particularly with human rhinovirus, and allergic sensitisation in young children in terms of asthma risk. However, knowledge of allergic sensitisation in early infancy is limited, as is the role of early allergic sensitisation in the development of recurrent wheeze and asthma. The potential interactions between specific respiratory viruses and early allergic sensitisation in the development of asthma are unclear.

The specific research aims were therefore:

1. To identify risk factors for receiving supportive care for acute, moderate to severe infant bronchiolitis.
2. To determine if clinical score or parental assessment using a visual analogue scale of acute infant bronchiolitis at the time of hospitalisation predicts the short-term prognosis
3. To determine if severity of acute bronchiolitis, defined by length of hospital stay, receiving supportive care, clinical score or parental visual analogue scale is associated with early asthma development.
4. To determine prevalence of early allergic sensitization and the role of allergic sensitisation, type or load of viruses or salivary cortisol during acute bronchiolitis in infancy for asthma development.

Methods

The Bronchiolitis ALL study, South-East Norway, is a multicentre, double-blind, randomized clinical trial, including 404 infants 0–12 months of age with acute bronchiolitis admitted to paediatric departments of eight different hospitals from January 2010 through Mai 2011. The infants were randomly selected to receive inhaled racemic adrenalin or inhaled saline on demand or at fixed scheduled intervals. In addition, 240 infants were recruited by letter from a general population to serve as controls. In this thesis, the control group was used only for paper #3, in the assessment of allergic sensitisation in infancy.

At study enrolment, the infants underwent a clinical examination and were scored by a bronchiolitis severity score. The parents underwent a structured interview and assessed the severity of their child on a three-item visual analogue scale (VAS), evaluating the child's activity level, interest in food and level of illness. Nasopharyngeal aspirates were collected on study entry using polymerase chain reaction (PCR) analyses for virus identification and viral loads at the University of Athens at the end of the study inclusion. Serum Immunoglobulin E (IgE) were analysed from blood samples, taken at inclusion, at Fürst Medical Laboratory, using ImmunoCAP by Phadia AB, Uppsala, Sweden. The blood samples with Phadiotop Infant® of at least 0.15 kilo unit per litre (kU/l) (n=89) were further analysed for specific IgE to hens' egg, cow's milk, peanut, cat, dog, birch, timothy and house dust mite, specified down to 0.10 kU/l. Salivary cortisol was sampled on the first morning after hospital admission and analysed at Karolinska Institutet, Stockholm, by radioimmunoassay.

The number of episodes of bronchial obstructions were registered at study inclusion and at the two-year follow-up investigation, providing the basis for defining recurrent wheeze (at least three episodes) used as a proxy for asthma.

Results

Among the 404 infants hospitalised with acute bronchiolitis, 51% received supportive care during the hospital stay; 166 (41%) received oxygen therapy, 116 (29%) received nasogastric tube feeding and 30 (7%) received ventilatory support by CPAP.

Background and clinical characteristics at study enrolment with the greatest Odds Ratio (OR)

(95% confidence interval (CI)) for receiving supportive care in multivariate logistic regression analyses were: Caesarion section delivery 2.10 (1.20, 3.67), peripheral capillary oxygen saturation (SpO_2) < 92% 4.65(1.52, 14.24), heart rate 1.01 (1.0, 1.03) and age at hospitalisation 1.0 (0.99, 1.0).

The clinical score and the three parental VAS- items; activity level (Activity), interest in food (Feeding) and how ill is the child? (Illness) at enrolment were all significantly associated with receiving supportive care with OR of 1.28 (1.04, 1.56), 1.26 (1.15, 1.39), 1.23 (1.13, 1.34) and 1.36 (1.18, 1.56) respectively. The parental VAS item Illness was most strongly associated with receiving supportive care, providing positive and negative likelihood ratios of 2.1 and 0.55, and sensitivity and specificity of 61% and 71% respectively. The clinical score provided positive and negative likelihood ratios of 1.07 and 0.91, and sensitivity and specificity of 59% and 45 % respectively.
Length of Stay was found to be linearly dependent on the clinical score and parental VAS- items Aktivity and Illness on a cubic scale with $\beta_{\text{Clinical score}}$ (95%CI) 3.18 (2.59, 3.77), p= 0.007, β_{Activity} (95%CI) 3.67 (3.38, 3.96), p= 0.005 and β_{Illness} (95%CI) 3.41 (2.95, 3.87), p= 0.005.

There was no significant association between severity assessment of acute bronchiolitis and asthma development.

Allergic sensitisation was assessed in 368 infants hospitalized with acute bronchiolitis and in 224 infants in the control group. The overall mean age (range) was 5.1 (0.2- 13.6) months. We found allergic sensitisation to any allergen in 8.5% of the infants, with similar rates among infants from the bronchiolitis and control group. The infants were most commonly sensitised to food allergens (7%);

egg (4%), cow's milk (4%) and peanut 1%. Only 2% were sensitised to inhalant allergens, cat being most common.

Of the 294 children attending the two-year follow-up, 143 (49%) had recurrent wheeze. We found no significant associations between bronchiolitis severity and early asthma development by two years of age.

Comparing children with and without recurrent wheeze at two years of age, RSV was detected in 83% versus 82% and HRV detected in 35% in both groups. In children with recurrent wheeze, 7% were sensitised in infancy, while 9% of children with no recurrent wheeze were sensitised. We found no significant associations between RSV or HRV, high viral load, allergic sensitisation or morning salivary cortisol level during acute bronchiolitis in infancy and recurrent wheeze at two years of age.

Discussion

Caesarion section delivery, SpO_2, heart rate and age at hospitalisation were found to increase the risk for receiving supportive care during hospital stay.

Caesarean section delivery is previously described to predispose for respiratory complications later in childhood.

Peripheral capillary oxygen saturation <92% at the time of hospitalisation were significantly associated with the risk of receiving supportive care during hospital stay. Measures of SpO_2 have been used since the 1980ies and included in several severity scores. There is no agreement on which level of SpO_2 to use in the clinical decision making in infants with acute bronchiolitis.

High RSV load increased the risk of supportive care during hospital stay, in line with previous studies describing high RSV load as associated with respiratory failure and severity of bronchiolitis.

Infants receiving inhalation therapy on demand had significantly shorter hospital stay, and less frequent oxygen therapy compared to infants receiving inhalations on fixed schedules. Providing

inhalations on demand may be less stressful and gives the infant more rest. Inhalations of racemic adrenaline was not superior to inhalations of saline regarding length of stay and use of supportive care. In a recent systemic review and meta-analysis, nebulised saline is suggested to be an active treatment for acute bronchiolitis, but more research is needed to confirm or refute this.

Parental severity assessments of acute bronchiolitis by VAS at hospital admission were significantly associated with LOS and use of supportive care during hospital stay. This novel finding emphasizes the importance of parental involvement in clinical decision making. Including parental evaluation in a standardised clinical evaluation may enhance the quality of the clinical decision making.

The lack of significant association between bronchiolitis severity and early asthma development in our study is in contrast to previous studies showing a strong relationship between bronchiolitis severity and asthma development. However, severity of bronchiolitis in other studies was defined as need for hospital admission, while all infants in this study cohort were hospitalised with moderate to severe bronchiolitis. This may indicate that the relationship between bronchiolitis severity and asthma development is not linear and is not affected by severity beyond needing hospital admission. Whether viral bronchiolitis in infancy or early childhood promotes asthma development or identifies infants at risk for subsequent wheezing is still unclear.

The observed rate of allergic sensitisation, mostly to food allergens in infants at a mean age of 5 months are in line with the Copenhagen Prospective Study on Asthma in Childhood ($COPSAC_{2000}$) study but lower than the Allergy Research Centre (DARC) study. Our study adds further insight to the limited knowledge about allergic sensitisation in infants younger than 6 months. The similar rates of sensitisation among infants from the bronchiolitis and control group may be explained by the low number of sensitised infants.

The risk of early asthma at two years of age was not increased by type of virus, early allergic sensitisation or salivary morning cortisol during the acute bronchiolitis. Our findings do not support a

role of specific viruses or early allergic sensitisation as important risk factors for recurrent wheeze at two years of age.

Conclusions

Caesarion section delivery, $SpO_2 < 92\%$, increased heart rate and lower age at hospitalisation were found to increase the risk for receiving supportive care during hospital stay.

The parental VAS item Illness was most strongly associated with receiving supportive care, with greater sensitivity and specificity compared to the clinical score.

We found no association between the severity of acute bronchiolitis in infancy and early asthma development.

Allergic sensitisation, specific viruses and viral load or salivary morning cortisol did not increase the risk of early asthma development in children hospitalised with acute bronchiolitis during first year of life.

1.3 Sammendrag på norsk

Bakgrunn

Akutt bronkiolitt er en viral nedre luftveisinfeksjon som primært dominerer i vinterhalvåret, ofte forårsaket av respiratorisk syncytial virus (RSV), men andre virus kan også være involvert. Øvre luftveis symptomer er vanligst de første dagene etter smitte. Deretter følger symptomer fra de nedre luftveier som hoste, rask pust, tungpustethet og jugulære, interkostale og subkostale inndragninger. De fleste barn har milde symptomer uten alvorlig pustebesvær. Noen vil trenge sykehusinnleggelse for oksygen tilskudd, sondeernæring eller pustestøtte via CPAP eller respirator. Akutt bronkiolitt er en av de hyppigste årsakene til sykehusinnleggelse hos spedbarn og barn under ett år.

Foruten støttebehandling ved spiseproblemer, lav oksygenmeting og/ eller alvorlige pusteproblemer, finnes ingen behandling for å avkorte sykdommens lengde og alvorlighetsgrad.

Det er utviklet flere skåringsmodeller for å kunne vurdere alvorlighetsgrad og effekt av behandling hos barn med akutt bronkiolitt. Disse kombinerer ofte objektive og subjektive parametere, men den kliniske nytteverdien, presisjon og pålitelighet er uklar. Foreldres evne til å vurdere barn med akutt bronkiolitt har ikke tidligere blitt studert.

Akutt bronkiolitt og allergisk sensibilisering er funnet å kunne øke risikoen for senere astmautvikling. Tidligere studier har vist en synergi mellom nedre luftveisinfeksjon, spesielt forårsaket av HRV, og allergisk sensibilisering hos små barn og risiko for astma utvikling. Kunnskapen om allergisk sensibilisering i spedbarnsalderen og dens rolle ved utvikling av wheeze og astma er begrenset.

Målsetning

De spesifikke forskningsspørsmålene var derfor:

1. Å identifisere risikofaktorer for å motta støtteterapi hos spedbarn med akutt moderat til alvorlig bronkiolitt.
2. Å bestemme om klinisk skår eller foreldres vurdering av barn med akutt bronkiolitt med visuell analog skala ved sykehusinnleggelse predikerer sykdomsprognose på kort sikt.
3. Å avgjøre om alvorlighetsgraden av akutt bronkiolitt, definert av lengden av sykehusoppholdet, bruk av støtteterapi, klinisk skåringsmodell eller foreldrevurdering med en visuell analog skala er assosiert med tidlig astmautvikling.
4. Å avgjøre prevalensen av tidlig allergisk sensibilisering og avgjøre om allergisk sensibilisering, type eller mengde virus eller spyttkortisol hos spedbarn med akutt bronkiolitt øker risikoen for astma utvikling.

Metode

Bronchiolitis ALL-studien, er en multisenter, dobbeltblind, randomisert klinisk studie, som inkluderte 404 spedbarn med akutt bronkiolitt i alderen fra 0 til 12 måneder innlagt på åtte forskjellige barneavdelinger i Sør-Øst-Norge, mellom januar 2010 og mai 2011. De ble tilfeldig trukket ut til å motta inhalert racemisk adrenalin eller inhalert saltvann ved behov eller ved faste intervaller. I tillegg ble 240 spedbarn fra en generell befolkning inkludert i en kontrollgruppe. I denne avhandlingen ble kontrollgruppen bare brukt i tredje publikasjon for å vurdere allergisk sensibilisering i spedbarnsalderen.

Ved inklusjon i studien ble det gjennomført en grundig klinisk undersøkelse og innleggende lege vurderte barnet med et klinisk skåringsverktøy. Et strukturert intervjue ble utført med en eller begge foreldre, som deretter vurderte hvor sykt deres barn var ved hjelp av en visuell analog skala (VAS) med tre elementer; aktivitetsnivå, spisevillighet og allmenntilstand. Nasofarynksaspirat tatt ved inklusjon ble analysert ved hjelp av polymerasekjedereaksjon (PCR) ved Universitetet i Aten etter at

alle pasienter var inkludert. Serum Immunoglobulin E (s-IgE) ble analysert fra blodprøver, tatt ved inkludering, ved Fürst medisinske laboratorium, ved hjelp av ImmunoCAP, Phadia AB, Uppsala, Sverige. Blodprøver med Phadiotop Infant® på minst 0.15 kU/l (n = 89) ble videre analysert for s-IgE til egg, kumelk, peanøtt, katt, hund, bjørk, timotei og husstøvmidd, spesifisert ned til 0.10 kU/l. Det ble tatt prøve av spyttkortisol morgenen etter innleggelse på sykehus som ble videre analysert med radioimmunoassay ved Karolinska Institutet, Stockholm.
Antall luftveis obstruktive episoder ble registrert ved studieinklusjon og ved to års oppfølging.

Resultater

Av 404 spedbarn innlagt på sykehus med akutt moderat til alvorlig bronkiolitt fikk 51% støttebehandling under sykehusopphold; 166 (41%) fikk oksygentilskudd, 116 (29%) fikk væske via nasogastrisk sonde og 30 (7%) fikk ventilasjonsstøtte med CPAP.

Bakgrunns data og kliniske parametere ved studieinklusjon med størst Odds Ratio (OR) (95% konfidensintervall (CI)) for å motta støttebehandling i multivariat logistisk regresjonsanalyse var keisersnitt 2.10 (1.20, 3.67), oksygenmetning (SpO_2) < 92% 4.65(1.52, 14.24), hjerte frekvens 1.01 (1.0, 1.03) og alder ved sykehusinnleggelse 1.0 (0.99, 1.0).

Den kliniske skåren og de tre foreldre VAS-elementene; aktivitetsnivå, spisevillighet og allmenntilstand, var alle signifikant assosiert med å motta støtteterapi med oddsratio på henholdsvis 1.28 (1.04, 1.56), 1.26 (1.15, 1.39), 1.23 (1.13, 1.34) and og 1.36 (1.18, 1.56). Foreldre VAS; allmenntilstand var sterkest assosiert med å motta støtteterapi med en positiv og negativ likelihood ratio på 2.1 og 0.55 og sensitivitet og spesifisitet på 61% og 71%. Den kliniske skåren hadde en positiv og negativ likelihood ratio på 1.07 og 0.91 og sensitivitet og spesifisitet på 59% og 45%.

Lengden av sykehusoppholdet ble funnet å være lineært avhengig av den kliniske skåren og foreldre VAS-elementene; aktivitetsnivå og allmenntilstand. Dette gav $\beta_{\text{Clinical score}}$ (95%CI) 3.18 (2.59, 3.77), p= 0.007, β_{Activity} (95%CI) 3.67 (3.38, 3.96), p= 0.005 and β_{Illness} (95%CI) 3.41 (2.95, 3.87), p= 0.005.

Allergisk sensibilisering ble vurdert hos 368 spedbarn innlagt på sykehus med akutt bronkiolitt og hos 224 spedbarn i kontrollgruppen. Gjennomsnitt alder var 5.1 (0.2- 13.6) måneder. Vi fant allergisk sensibilisering mot minst ett allergen hos 8.5% av spedbarna; 7% var sensibilisert mot minst ett matvareallergen (egg 4%, kumelk 4% og peanøtt 1%) og 2% var sensibilisert mot minst ett inhalasjonsallergen.

Blant de 294 barna som deltok på to-års oppfølgingen hadde 143 (49%) hatt 3 eller flere obstruktive episoder. Vi fant ingen signifikante assosiasjoner mellom bronkiolittens alvorlighet og tre eller flere obstruktive episoder, som en proxy for astma, ved to års alder.
Nå vi sammenlignet de barna med tre eller flere obstruktive episoder ved to års alder med de med færre, ble RSV påvist hos 83% mot 82% og human rhinovirus (HRV) påvist hos 35% i begge gruppene. Hos barn med minst tre obtruktive episoder ved to års alder var 7 % allergisk sensibilisert i spedbarnsalder, mens 9 % av barna med færre enn tre obstruktive episoder ved to års alder var allergisk sensibilisert i spedbarnsalder. Vi fant ingen signifikante assosiasjoner mellom RSV eller HRV, høyt antall virus kopier av HRV eller RSV, allergisk sensibilisering eller spytt-kortisolnivå om morgenen under akutt bronkiolitt i spedbarnsalder og minst tre obstruktive episoder ved to års alder.

Konklusjon

Å bli født med keisersnitt, SpO_2 >92%, økt hjerterytme og lav alder ved sykehusinnleggelse ble funnet å øke risikoen for støtteterapi under sykehusinnleggelse med akutt bronkiolitt signifikant.

Den kliniske skåren og de tre VAS skårene utført ved sykehusinnleggelse predikerte bruken av støtteterapi og lengden på sykehusoppholdet, foruten VAS- spisevillighet som bare predikerte bruk av støtteterapi.

Vi fant ingen signifikant assosiasjon mellom alvorlighetsgrad av akutt bronkiolitt, vurdert ved innleggelse og astmautvikling ved to års alder.

Allergisk sensibilisering, spesifikke virus, virus mengde og morgen spyttkortisol økte ikke risikoen for tidlig astma utvikling hos spedbarn <1 år innlagt på sykehus med akutt bronkiolitt.

1.4 Abbreviations

LRTI	Lower Respiratory Tract Infection
RSV	Respiratory Syncytial Virus
MPV	Metapneumovirus
AdV	Adenovirus
CoV	Coronavirus
HRV	Human Rhinovirus
IgE	Serum Immunoglobulin E
s-IgE	Specific Immunoglobulin E
PCR	Polymerase Chain Reaction
SpO_2	Peripheral capillary oxygen saturation
LOS	Length of Hospital Stay
OR	Odds Ratio
kU/l	Kilo Unit per Liter
COPSAC	The Copenhagen Prospective Study on Asthma in Childhood
DARC	the Danish Allergy Research Centre
SPT	Skin Prick Test
COAST	Childhood Origins of Asthma
RCT	Randomized Controlled Trial
VAS	Visual Analogue Scale

VAS- Activity	Activity level
VAS- Eating	Interest in food
VAS-Illness	How ill is the child?
CPAP	Continuous positive airways pressure
SD	Standard Deviation
CI	Confidence Interval
ROC	Receiver Operating Characteristic
AUC	Area under the receiver operating curve
RDAI	Respiratory Distress Assessment Instrument
AAP	The American Academy of Paediatrics
NICE	National Institute for Health and Care Excellence

1.5 List of papers

Paper #1:

Skjerven HO, Hunderi JO, Brugmann-Pieper SK, Brun AC, Engen H, Eskedal L, Haavaldsen M, Kvenshagen B, Lunde J, Rolfsjord LB, Siva C, Vikin T, Mowinckel P, Carlsen KH, Lodrup Carlsen KC. *N*

Racemic adrenaline and inhalation strategies in acute bronchiolitis

The New England Journal of Medicine 2013; 368:2286-93

Paper #2:

Hunderi JOG, Lødrup Carlsen KC, Rolfsjord LB, Carlsen KH, Mowinckel P, Skjerven HO.

Parental severity assessment predicts supportive care in infant bronchiolitis

Acta Paediatrica 2019 Jan;108(1):131-137.

Paper #3:

Skjerven HO, Hunderi JOG, Carlsen KH, Rolfsjord LB, Nordhagen L, Berents TL, Bains KES, Buchmann M, Carlsen KCL

Allergic sensitisation in infants younger than one year of age

Pediatr Allergy Immunol 2020 Feb;31(2):203-206.

Paper #4:

Hunderi JOG, Rolfsjord LBD, Lødrup Carlsen KC, Holst R, Bakkeheim E, Berents TL, Carlsen KH, Skjerven HO.

Virus, allergic sensitisation and cortisol in infant bronchiolitis and risk of early asthma

ERJ Open Res 2020; 6: 00268-2019

2 General introduction

2.1 Acute Bronchiolitis

Acute bronchiolitis is a lower respiratory tract infection (LRTI) (1) mainly affecting infants and young children (1-3) and is a common cause of hospitalisation (4, 5), especially during winter epidemics (6). In most European countries acute bronchiolitis is defined as a disorder in children <12 months (7), while the United States includes children aged <24 months (8). Hospitalisations with acute bronchiolitis are recognised as a major health burden worldwide (9, 10). In a Norwegian study conducted from 1993 to 2002, 2.2% of children <1 year of age were hospitalised with respiratory syncytial virus (RSV) bronchiolitis (6). In the United States 3.9% of all infants ≤ 5 months of age were hospitalised with RSV bronchiolitis between 1997 and 2006 (11).

Acute bronchiolitis is a clinical diagnosis defined by rapid respiration, dyspnoea, wheezing, chest recession, cough, rhonchi and rales (1). The bronchiolitis course is highly variable (12); usually mild and manageable at home, but can result in a need for hospitalisation and may be life threatening in some infants (9, 13).

The most common pathogen of acute bronchiolitis in infants is RSV (14-16), followed by Human metapneumovirus (MPV), Para influensa virus, adenovirus (AdV), influensa virus, coronavirus (CoV) and enterovirus (13, 14, 16, 17). Human rhinovirus (HRV) is commonly identified in children with LRTI infection and bronchiolitis, but is more prominent with increasing age (>12 months of age) (17-20).

After virus being transmitted via direct contact or by aerosol particles and replicated in epithelial cells of the airways (18), inflammatory responses are initiated in the airway epithelium causing necrosis and cilial damage (21). After an incubation period of 4 to 6 days, upper respiratory tract symptoms usually dominate, often accompanied by fever and poor feeding (12). Epithelial oedema, augmentation of mucus and widespread airway occlusion is found with increasing severity, causing

atelectasis and hyperinflation (8, 12, 21, 22). Because of this clinical course some infants develop feeding difficulties and breathing problems with or without oxygen desaturation.

Premature birth, chronic lung disease of prematurity, congenital heart diseases and immunodeficiency are known risk factors for a more severe bronchiolitis (8, 12). In previously healthy infants, young age is the single most important risk factor for developing a more severe bronchiolitis (3, 23). Male gender and having older siblings are also identified as risk factors for hospitalisation (24). However, most infants hospitalised with bronchiolitis have no known risk factors (3).

There are no well-defined hereditary factors for acute bronchiolitis (18). It is not clear whether parental atopy predisposes for a more severe bronchiolitis. In a quantitative review, Kneyber and co-workers found no significant associations between infant or parental atopy and acute RSV bronchiolitis (25), defining atopy by allergic rhinitis, urticaria or rashes in response to antigens (food, inhalation or drug) and/ or elevated serum Immunoglobulin E (IgE) level. On the other hand, in a population-based, retrospective cohort study of more than 100 000 term infants in Tennessee, USA, enrolled between 1995 and 2003, infants with maternal asthma were more likely to have bronchiolitis diagnosis than infants of mothers without asthma (10).

In the Bronchiolitis ALL study, in which infants had a mean age of 4 months, multiple viruses were found in 61% of the infants hospitalised with acute bronchiolitis, not affecting disease severity (16). This is in line with a Dutch study describing that multiple viruses were detected in 41% of the children with bronchiolitis, not associated with disease severity (26). In contrast, only 15% of infants < 2 years (mean 8 months) of age were found positive to multiple viruses in a Finnish prospective multicentre study (27), analysing for RSV, HRV, parainfluenza virus, influenza virus, MPV and CoV with subtypes by polymerase chain reaction (PCR) assays.

A more severe bronchiolitis is described in the Bronchiolitis ALL study and others in relation to high RSV genomic load (16, 28), but not found for HRV genomic load (16, 29). The viral aetiology detected

in the Bronchiolitis ALL study (16), led to further research questions providing the bases for the fourth publication included in this theses (30).

2.2 Severity assessment and management

Due to the variable course of bronchiolitis it may be challenging to determine which infants are at risk of severe disease that may lead to severe respiratory impairment (12). Hospitalisation should be considered when age is less than 3 months, inadequate ability to feed, seriously reduced general condition, severe respiratory distress, persistent peripheral capillary oxygen saturation (SpO_2), less than 92- 90%, observed or reported apnoea, premature birth or other underlying medical conditions (13, 31). However, the decision-making is affected by the experience of the attending physicians (32), the distance between the hospital and home (13) and the calculated risk of complications and need for supportive care (12, 33-35).

Both general severity scores (36-38) and bronchiolitis severity scores (39-42) have been developed with the intention to assess severity of disease, the effects of treatment or to identify children at risk of severe disease impairment **(Table 1)**. These combine objective and subjective parameters such as heart rate, respiratory rate, SpO_2, chest retractions and wheezing to predict hospital admission (32, 43-45) and LOS (32, 44) as a proxy for disease severity. However, the current scoring systems validity, reliability and utility are questioned to allow for clinically meaningful use in children with acute dyspnoea or wheeze (46) or to predict a deterioration of the bronchiolitis (14).

Parental empowerment and collaboration with healthcare providers is increasingly emphasized in clinical decision-making (47, 48). However, there are few studies determining the accuracy and predictive ability of parental evaluation of disease severity of their child.

Table 1 *Scoring tools assessing bronchiolitis severity (49)*

Instrument	Author	n Age (months)	Measured Characteristics	Validity
Tal Scoring system	Tal A. et.al (39)	32 range; 1- 12 months	- Respiratory Rate - Wheezing - Cyanosis - Accessory respiratory muscle utilization	AUC (95% CI) 0.69 (0.13, 1.0) for predicting oxygen requirement at 12 and 24 hours (50)
Modified- Tal Scoring system	De Boeck K et.al (51)	29 median; 194 days	- Respiratory Rate - Wheezing - Oxygen saturation - Accessory respiratory muscle utilization	AUC (95% CI) 0.75 (0.34, 1.0) for predicting oxygen requirement at 12 and 24 hours (50)
The respiratory distress assessment instrument (RDAI)	Lowel DI et.al (40)	30 range; 0- 24 months	- Wheezing - Retractions - Respiratory rate	AUC 0.51 predicting hospital admission (52)
Modified respiratory distress assessment instrument (RDAI)	De Brasil D et.al (53)	84 mean; 3.5 months	- Wheezing - Retractions - Location of wheezing	Not Assessed
The Wang respiratory score	Wang EE et.al (41)	56 range; 0- 24 months	- Respiratory Rate - Wheezing - Retractions - General Condition	Significant negative correlation between the score and SpO_2 for two observers (41, 54)
A modified score of Wang	Beck R et.al (55)	27 range; 2- 12 months	- Wheezing - Retractions - Oxygen saturation - Respiratory rate - Heart rate	Not Assessed
The Kristjansson respiratory score	Kristjansson et.al (42)	34 range; 0- 18 months	- Respiratory rate - Chest recession - Breath sound - Skin color - General condition	Significant negative correlation between the score and SpO_2 for two observers (54)
Severity Score	Wainwright C et.al (56)	194 range; 0- 12 months	- Respiratory effort - Oxygen saturation - Respiratory Rate	Not Assessed
A Bronchiolitis severity assessment tool	Walsh P et.al (32)	99 range; 0- 24 months	- Retractiona - Heart rate - Age - Dehydration	91% sensitivity and 83% specificity for predicting hospital admission (32)
The respiratory score	Gajdos V et.al (57)	180 range; 0- 18 months	- Age based respiratory rate - Retraction signs - Wheezing	Not Assessed
A respiratory clinical score	Liu LL et.al (58)	55 range; 1 month- 19 years	- Respiratory rate - Retractions - Dyspnea - Auscultation	Not Assessed
Bronchiolitis risk of admission score	Marlais M et.al (45)	449 range; 0- 12 months	- Duration of symptoms - Respiratory rate - Heart rate - Oxygen saturation - Age at presentation	AUC (95% CI) 0.81 (0.77, 0.85) for requiring admission with sensitivity of 74% and specificity of 77% (45)

AUC- Area under the receiver operating curve, SpO_2 - Peripheral capillary oxygen saturation

Data from: Rodriguez-Martinez CE, Sossa-Briceño MP, Nino G. Paediatr Respir Rev. 2018 Jan;25:43-57.

The hospital management of acute bronchiolitis is mainly supportive (12), including oxygen therapy, nasogastric tube feeding and ventilatory support (8, 13, 15, 59). Most previous studies have failed to show any benefit from specific treatment to reduce severity or to shorten the bronchiolitis course (8, 60). At the time of establishing the Bronchiolitis ALL study, there was no widely accepted consensus in bronchiolitis treatment (59).

Adrenergic bronchodilators such as racemic adrenalin was commonly used in Scandinavia and North America, (59, 61, 62). Inhaled racemic adrenaline has previously been described as having a potential mucosal de-swelling effect (63) with only modest side effects such as tachycardia, discomfort and tremors (64, 65). In Norway, prior to the results of the Bronchiolitis ALL study randomised trial, reported in 2013 (66), inhalations with racemic adrenaline as frequently as every third to every hour were recommended for all infants hospitalised with bronchiolitis (67). Inhalation therapies were mostly given at fixed schedules and there was a lack of awareness of inhalation therapies on demand.

In a study by Kristjánsson et al. including 34 children aged less than 18 months, treatment with inhaled racemic adrenaline was found to improve oxygenation and clinical signs (42), also described in a Finnish study including 100 children, aged less than 48 months, hospitalised with bronchiolitis (68). A study by Sanchez et al. of 24 children less than 12 months of age with acute bronchiolitis, found a decrease in airway resistance after inhalation with racemic adrenaline (69). In 16 children with acute bronchiolitis, mean age 7.9 months, Lødrup Carlsen et al. described a significant improvement of lung function after inhalation with racemic adrenaline (70). Later a North- American study also found that inhalation with racemic adrenaline relieved respiratory distress, but did not shorten hospital stay (71). A study from United Kingdom, including 85 infants (1 month to 12 months of age), found no significant differences in admission rate, clinical score , respiratory rate, heart rate and SpO_2 comparing nebulized adrenaline to saline inhalations (72). A Canadian study including 149

children aged <12 months found no difference in length of stay comparing inhalations with epinephrine, albuterol and saline (73).

The results from the first larger multicentre randomized controlled trial comparing inhalations with nebulized single-isomer epinephrine with placebo (saline), conducted in Australia, were published in 2003 (56). This study, including 194 children younger than 12 months, found no significant reduction in length of hospital stay using inhaled epinephrine compared to saline.

Other treatment modalities such as leukotriene antagonists, antibiotics and inhaled hypertonic saline were shown not to improve the bronchiolitis course (31). Still, clinical practice varied because of controversy, confusion, and lack of evidence over the best treatment (15). At present in 2020 based upon recent studies including the Bronchiolitis ALL study (66), major guidelines recommend supportive treatment only for acute bronchiolitis (74).

With the lack of curative therapy, the main treatment option is supportive (12). We then need to know more about clinical features and factors predicting severity in infant bronchiolitis; who are most likely to deteriorate requiring supportive care, as a proxy for disease severity?

2.3 Prognosis, allergic sensitisation and asthma development

Bronchiolitis in infancy has been shown to increase the risk of recurrent wheeze and asthma development (75-78). It is not clear whether infant bronchiolitis initiates asthma development or whether moderate to severe bronchiolitis in infancy reveals children with predisposing factors (15, 76). Both RSV and HRV infections in early life are associated with recurrent wheeze and asthma (75, 77, 78). In a Swedish study of children <1 years of age hospitalised with RSV bronchiolitis, with an age- and gender-matched control group, 39% of the children with RSV were found to have current asthma at 18 years of age, compared to 9% in the control group, $p<0.001$ (78). Lemanske and co-workers found that at least 1 wheezing illness with RSV or HRV during infancy was associated with preschool wheeze with odds ratio (OR) of 3.0 and 10.0, respectively (77). As children grow older HRV is more often identified during LRTI leading to wheeze or asthma exacerbations, compared to the highly prevalent RSV among the youngest infants (16, 17, 20). Although high RSV genomic load has been associated with disease severity in terms of increased length of stay (LOS) and use of supportive care; oxygen and fluid supplement and ventilatory support (16, 28), the association between viral load and recurrent wheeze and asthma is not described.

Allergic sensitisation is usually documented by specific IgE (s-IgE) to food and/or inhalant allergens, commonly using s-IgE ≥0.35 kilo- unit- per- litre (kU/l) (79) and or skin prick test (SPT) with a wheal ≥3 mm (80) as cut-off levels for positive or negative tests in older children (81). However, recent studies have defined an SPT of at least 2 mm larger than the negative wheal as positive allergic sensitisation in children younger than two years of age (82-84) while the role of s-IgE levels lower than 0.35 kU/l is unclear . Allergic sensitisation may and may not be associated with symptoms after exposure to the culprit allergen (85), highlighting the importance of determining the clinical relevance of allergic sensitisation.

The few reports on allergic sensitisation in infants younger than 12 months are divergent (79, 83, 86), and there is lack of knowledge about the development of s-IgE sensitisation in early life. The Danish

Allergy Research Centre (DARC) birth cohort reported allergic sensitisation by s-IgE in 12.5% of 352 3-months old infants; 4.3% to food and 9.4% to inhalant allergens (86), with 16.7% being sensitised at 6-months of age; 8.5% to food and 9.1% to inhalant allergens (86). The Copenhagen Prospective Study on Asthma in Childhood ($COPSAC_{2000}$) high-risk cohort (83), assessing 6 months old infants, found 7.8% to be sensitised by s-IgE to food allergens and 0.6% to inhalant allergens while a Swedish study reported 11% allergic sensitisation overall in the same age group (79). This Swedish study reported also low allergic sensitisation, defined as s-IgE 0.1- 0.34 kU/l, in 5% to egg, 14% to milk and 4% to peanut in the 6-months- old- infant (79).

Allergic sensitisation has been reported to increase the risk of recurrent wheeze and asthma (87-89), with an apparent synergistic effect in young children who also have a LRTI (90, 91). In the general population-based DARC birth cohort study a significant association between allergic sensitisation against food at 6 months of age and asthma at 6 years of age is described (86). The high-risk Childhood Origins of Asthma (COAST) birth cohort study found that HRV LRTI, but not RSV LRTI, increased the risk of recurrent wheeze in children who were sensitised to aeroallergens, assessed annually first six years of life (91), while Kusel and co- workers found that both RSV and HRV LRTI first year of life in sensitised children increased the risk of persistent wheeze at 5 years (90). However, there is a lack of studies assessing if such potential synergies between allergic sensitisation and HRV- or RSV LRTI (acute bronchiolitis) during infancy may affect the risk of early asthma development.

Asthma, a common chronic inflammatory airway disease associated with airway hyperresponsiveness, is characterized by recurrent episodes of wheeze, cough, breathlessness and/ or chest tightness (92, 93). The clinical symptoms of asthma are non-specific and variable in younger children, with wheeze being the most common symptom (94).

There are different patterns of childhood wheeze, and several different phenotypes have been recognized, although children may move between phenotypes during childhood (94, 95). In 2008, the European Respiratory Society classified early childhood wheeze as either episodic wheeze or

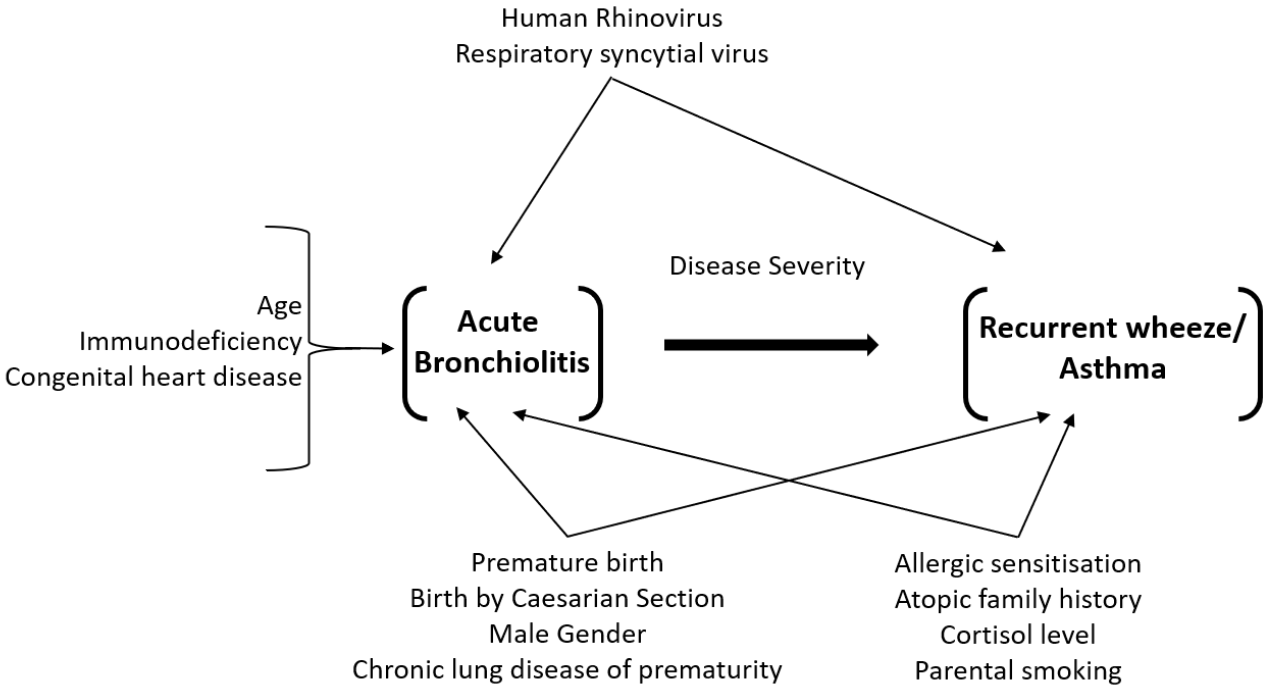
multiple-trigger wheeze, with asthma more common among those with multiple-trigger wheeze (93). Later an European Respiratory Society consensus group agreed that the distinction between episodic wheeze and multiple-trigger wheeze was not as clear-cut as previously postulated and that the wheeze patterns vary over time and with treatment (95).

Some children have viral-induced wheeze caused by narrow airways obstructed by mucus and oedema (96, 97), while others may have eosinophilic airway inflammation, particular found in atopic children (98). Due to the lack of appropriate asthma definition in children younger than five years of age, and as asthma may manifest in early childhood, we chose to use a proxy for asthma in two-year-old children based on a history of at least three episodes of wheeze.

Acute stress promotes the release of corticosteroids from the adrenal cortex (99). In the bronchiolitis ALL study salivary morning cortisol levels were higher in infants with acute bronchiolitis compared to a control group (100). This was in line with a study by Pinto et al. who demonstrated a higher level of plasma cortisol during acute RSV bronchiolitis compared to noninfected controls, with a higher cortisol level in infants with more severe disease (101). On the other hand, reduced cortisol level is shown in children with allergy and asthma, linked to impaired activity in the hypothalamic-pituitary-adrenalin axis (102, 103). In a mouse model, Forsyth and co-workers found opposing effects of short- and long-term stress on airway inflammation (104), postulating that chronic stress can exacerbate the chronic inflammatory responses of the airways. The role of cortisol level during acute bronchiolitis and later asthma development is not well studied.

With the likely multi-factorial triggering of asthma development, it is not clear what role each of the potential risk factors may play; these risk factors are specific viruses, high viral load of RSV or HRV, allergic sensitisation, morning salivary cortisol or interactions between these in infants with acute bronchiolitis **(Figure 1)**.

Figure 1 *Potential and known risk factors for acute bronchiolitis and for later recurrent wheeze and asthma development*



2.4 Objective and aims

The overall objective of this thesis was to provide further insight into management and prognosis of acute bronchiolitis and risk of asthma development in infants with acute bronchiolitis.

The specific research aims were therefore:

1. To identify risk factors for receiving supportive care for acute moderate to severe infant bronchiolitis. (paper #1, 2)
2. To determine if clinical score or parental assessment using a visual analogue scale of acute infant bronchiolitis at the time of hospitalisation predicts the short-term prognosis. (paper #1, 2)
3. To determine if severity of acute bronchiolitis, defined by length of hospital stay, receiving supportive care, clinical score or parental visual analogue scale is associated with early asthma development. (paper #2, 4)
4. To determine prevalence of early allergic sensitization and the role of allergic sensitisation, type or load of viruses or salivary cortisol during acute bronchiolitis in infancy for asthma development. (paper # 2, 3, 4)

3 Methods and subjects

3.1 Study design

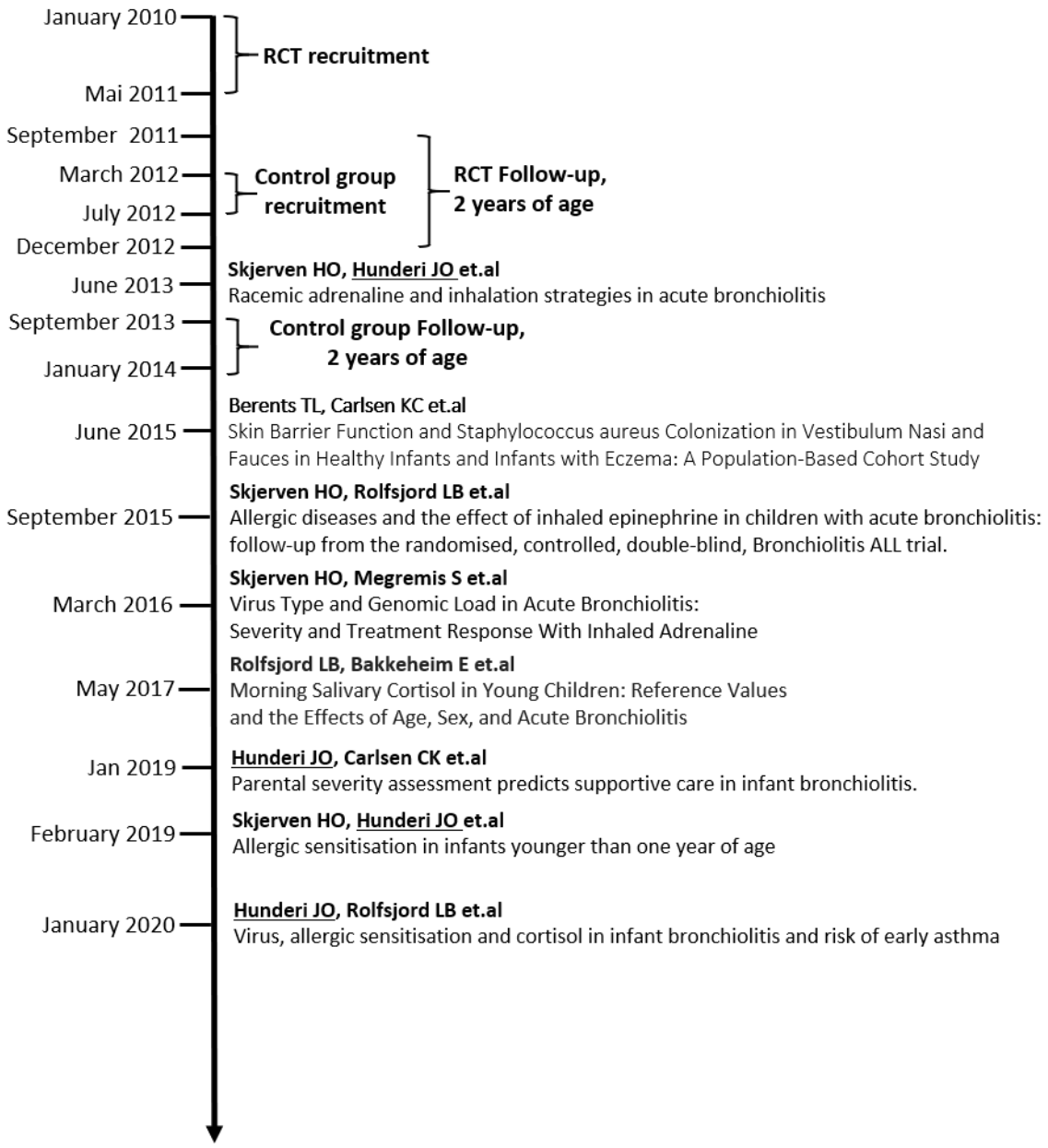
This thesis is based on the Bronchiolitis ALL study (ClincialTrials.gov number, NCT00817466. EudraCT number, 2009-012667-34), SE-Norway trial that consists of two parts; a randomized controlled trial (RCT), including infants with acute bronchiolitis – hereafter termed the bronchiolitis group – and an exploratory prospective observational study, including the bronchiolitis group as well as a control group recruited from the general infant population **(Figure 2)**. Both groups underwent a follow-up study at 2 years follow-up. The study design is shown in **Figure 3**.

The clinical trial, conducted from January 2010 through May 2011, was a randomised double blinded factorially designed 2-by-2 multicentre study including infants admitted to hospital due to moderately-to-severely acute bronchiolitis.

The control group recruited from the general infant population was used in the prospective, observational studies.

The two-year follow-up investigations included both the bronchiolitis group and control group and provided data for the prospective observational study **(Figure 3)**.

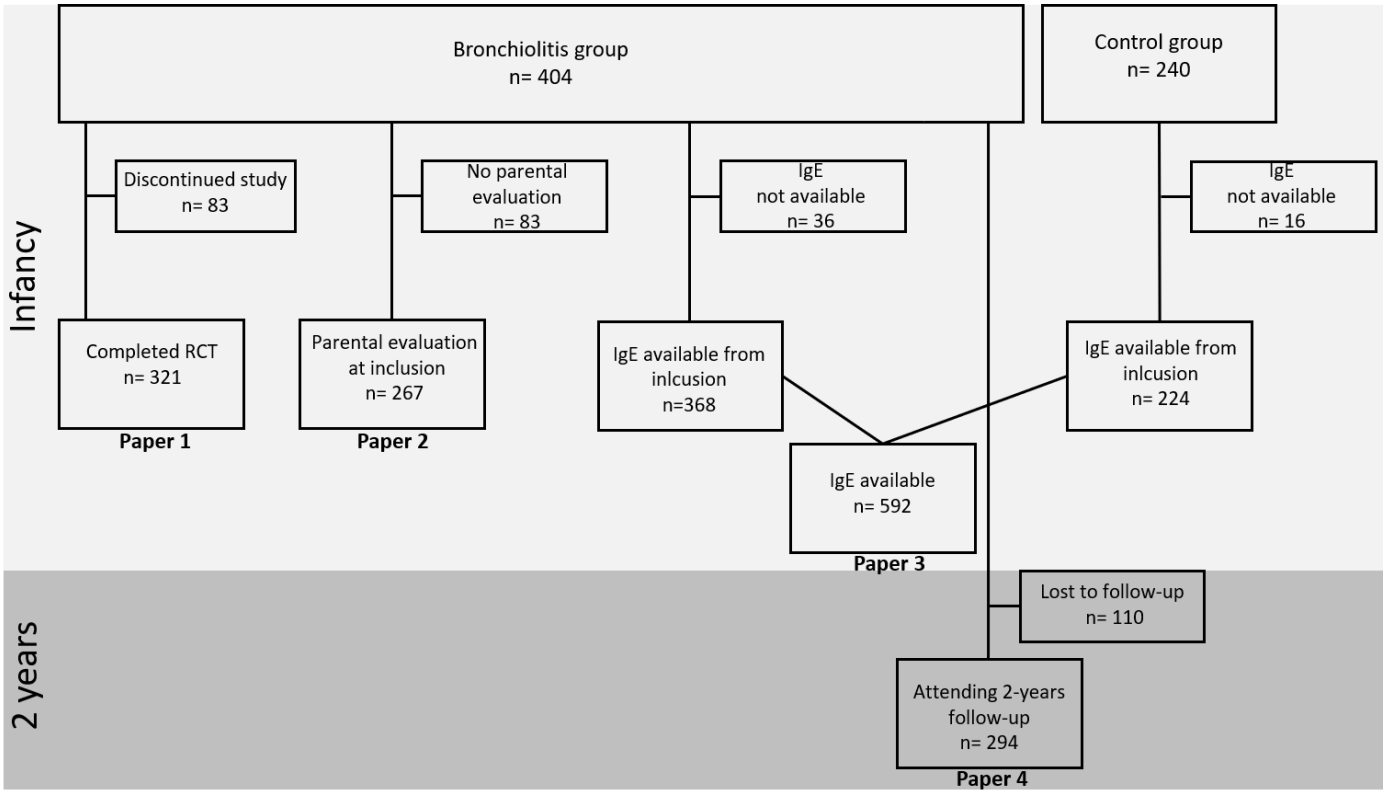
Figure 2 *Timeline of the Bronchiolitis ALL study with relevant publications for this PhD-thesis*



Papers 1 and 2 are based on the 404 infants recruited into the RCT. While Paper 1 determined the efficacy of racemic adrenaline on LOS and use of supportive care, Paper 2 evaluated the predictive value of parental severity evaluation at the time of hospitalisation and use of supportive care during hospital stay.

Paper 3 included all infants from the bronchiolitis and control groups with available s-IgE at enrolment for determining the rate of early allergic sensitisation. **Paper 4** explored in prospective observational analyses the risk of developing asthma among all infants in the bronchiolitis group who attended the two-year follow-up investigations **(Figure 3)**.

Figure 3 *Flowchart of the study cohort*



The Inclusion criteria in the bronchiolitis group were below 12 months of age, clinical signs of bronchiolitis, as defined by Court (1) and a clinical score (42) of at least 4 on a scale from 0-10, 10 indicating most severe disease **(Table 2)**.

Table 2 *Clinical Score*

	Score 0	**Score 1**	**Score 2**
Respiratory rate (breaths/min)	<40	40-60	>60
Respiratory Chest recessions	none	Moderate Costodiaphragmatic	Severe. As 1+ rib and jugular retractions
Auscultatory breath sounds	Vesicular	Wheeze, rales/ronchi	Faint ± severe wheeze ± pronounced rales and rhonchi
Skin colour	Normal	Pallor	Cyanosis
General Condition	Not affected	Moderately affected	Severely affected

The clinical score was completed by doctors at inclusion and daily during hospital stay. A clinical core ≥four of ten was required for study inclusion. The score is identical to that used in a study of acute bronchiolitis by Krisjansson et al (42).

The inclusion criterion for the control group was age 12 months or younger at the time of recruitment.

The exclusion criteria in the bronchiolitis group were severe underlying disease (cardiac, pulmonary (other than obstructive airways disease), immunological, neurological or oncological disease), more than one previous episode of obstructive airway disease, more than four weeks persisting lower airway symptoms (e.g., coughing), and use of inhaled or systemic steroids in the last four weeks.

Exclusion criteria for the control group were any cardiac, pulmonary (other than obstructive airways disease), immunological, neurological or oncological disease.

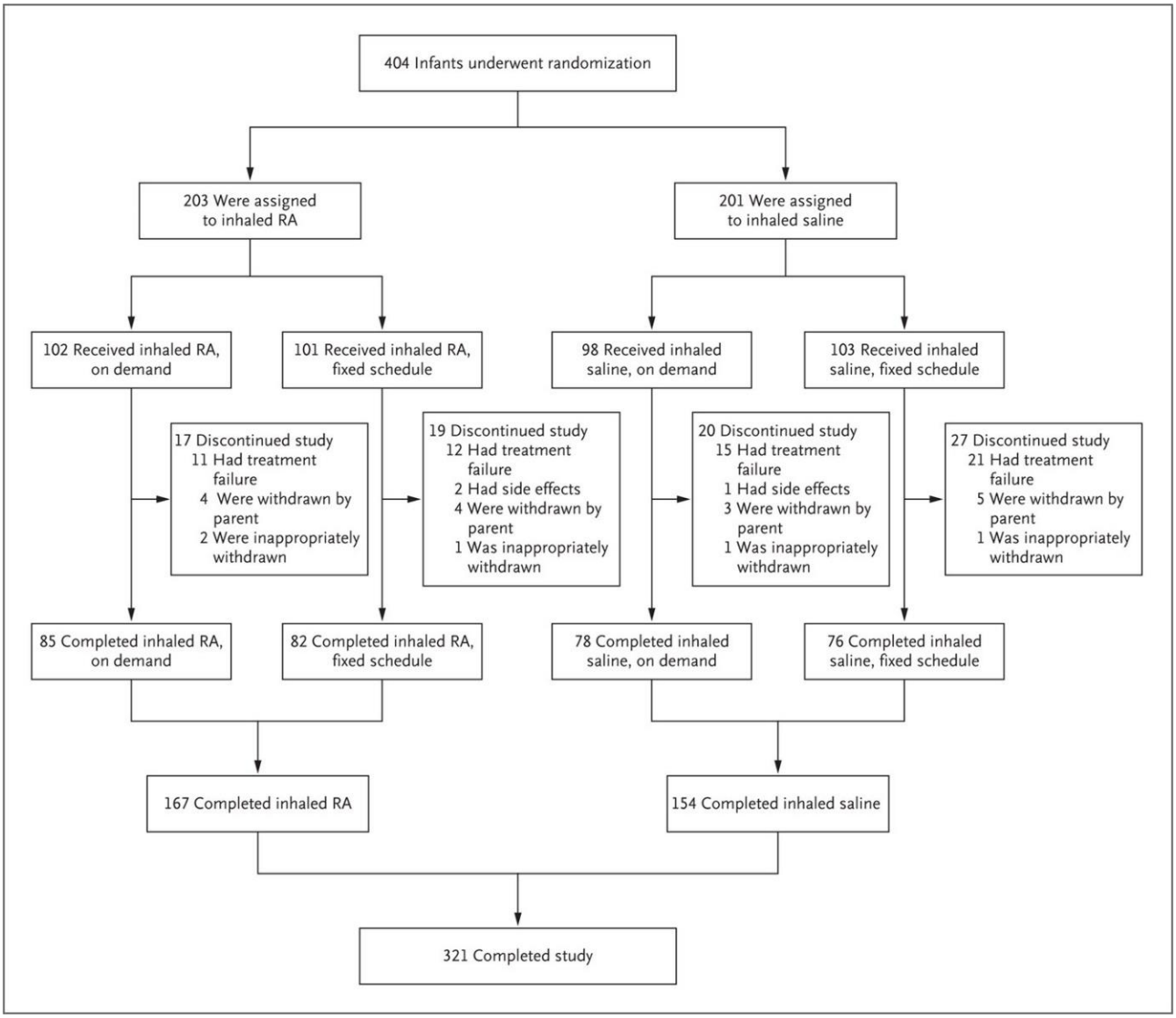
Recruitment: In the RCT infants with acute bronchiolitis fulfilling inclusion and exclusion criteria, were recruited when admitted to one of the eight participating hospitals in South-Eastern Norway Regional Health Authority;

- Innlandet Hospital Trust; Elverum and Lillehammer
- Vestre Viken Hospital Trust; Drammen
- Vestfold Hospital Trust
- Telemark Hospital Trust
- Sørlandet Hospital Trust; Kristansand
- Oslo University Hospital Trust
- Østfold Hospital Trust

The control group was recruited through a letter of invitation sent to the parents of 3000 infants, randomly selected from the general population register in the cities of Oslo and Fredrikstad, both in south-eastern Norway. Of these, 240 responded and were enrolled. The infants underwent clinical examination and blood sampling within three months beginning in March 2012.

Randomisation: Infants recruited in **the bronchiolitis group**, were randomly assigned to receive inhalations of racemic adrenaline or isotonic saline. The two groups were further randomized to different inhalation strategies: on demand or on fixed schedules. **(Figure 4)**.

Figure 4 *Randomization of the Study Patients in the RCT (Paper 1)*



In five children, the study medication was discontinued because of the following administrative failures: administration of open inhaled racemic adrenaline, suspected pertussis infection, delayed biologic sampling, administration of a dose of the study medication that was too high, and insufficient supply of study medication.

Reproduced with permission from New England Journal of medicine Skjerven HO, Hunderi JO, Brugmann-Pieper SK, et al. Racemic adrenaline and inhalation strategies in acute bronchiolitis, 368 (24), 2286-93. Copyright © (2016) Massachusetts Medical Society.

The two study medications: 10 ml of racemic adrenaline dissolved in 0.9% saline to form a solution of 29 mg per millilitre or 0.9% saline alone, were prepared in identical bottles, labelled with a numerical code indicating the type of medication and administration; on demand or on a fixed schedule. The dose administered was based on the infant's weight: 0.1ml for an infant weighing less than 5Kg, 0.15

ml for those weighing 5 to 6.9 kg, 0.2 ml for those weighing 7 to 9.9 kg, and 0.25 ml for those weighing 10 kg or more. Medication was diluted in 2 ml of saline before nebulization and was administrated through a Sidestream Reusable Nebulizer with a Respironics Facemask (Both from Philips Respironics), driven by 100% oxygen at a rate of 6 litres per minute.

The randomization was executed with the use of SAS software, version 9.3. Centrally in blocks of eight, the infants were assigned to one of the four study groups. The study statistician communicated the randomization codes directly to the pharmacy, which prepared the study medication. The study centres were provided with a list of study numbers for use when assigning medication to enrolled infants and were not aware of the randomization block size.

All children from the bronchiolitis and the control groups were invited to attend the **two- years-of-age follow-up visit** by letter of invitation and/ or phone call to the caregivers of the participating infants. The two-years-of-age follow-up visit was performed for the bronchiolitis group from September 2011 through December 2012 and for the control group September 2013 through January 2014.

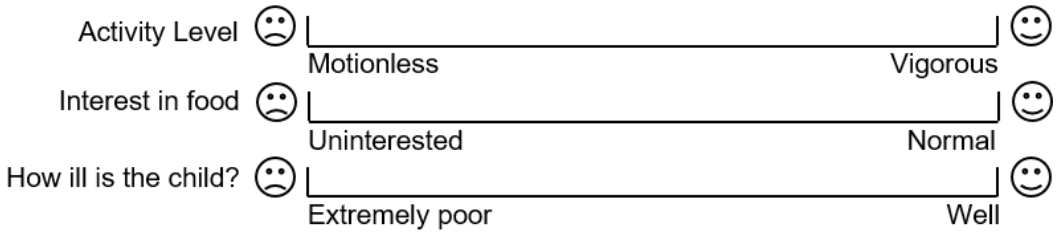
3.2 Methods

At hospital admission, after written informed consent was obtained from at least one parent, the infant was enrolled in the study. The physician enrolling the patient performed a structured parental interview, including past medical history, parental medical history and socio- demography. The infants underwent a clinical examination and biological specimens were collected from the nasopharynx, blood, urine and saliva. Additionally the infant was scored by a bronchiolitis severity score **(Table 2)** (42) performed by the physician and evaluated by the parents by a visual analogue scales (VAS) **(Figure 5)**. The physicians enrolling the patients were trained at investigator meetings as well as on site by Principal investigator and local primary investigators.

3.2.1 Bronchiolitis severity assessment

Infants were required to have a bronchiolitis severity score of at least 4 out of 10 **(Table 2)** to comply with the inclusion criterion (42). The assessment was performed by the enrolling physician. Before inhalation therapy with "study medication", the infant was evaluated by the parents by a VAS **(Figure 5)** scoring disease severity. The assessment consisted of a three-item smiley VAS on a 10-centimetre single horizontal line where 10 indicates most severe, as outlined to the left in **Figure 5**. The three categories concerned the activity level, hereafter termed Activity, the interest in food, hereafter termed Feeding, and the question "How ill is the child?", hereafter termed Illness.

Figure 5 *Parental VAS**,** assessing severity at acute infant*



The parental assessment consisted of a three-item smiley visual analogue scales (VAS) on a 10-centimeter single horizontal line where 10 indicates most severe. The three categories concerned the activity level termed Activity, the interest in food, termed Feeding and finally the question
"How ill is the child?" termed Illness.

From Acta Paediatrica, Gjengsto Hunderi JO, Lodrup Carlsen KC, Rolfsjord LB, Carlsen KH, Mowinckel P, Skjerven HO. Parental severity assessment predicts supportive care in infant bronchiolitis, Jan;108(1):131-13. Copyright © (2019).

3.2.2 Virus sampling and detection

Nasopharyngeal aspirates were collected at study inclusion by trained paediatric nurses using a standardized procedure with a tracheal suction set (Unomedical A/S, Lejre, Denmark). The sample was immediately frozen at – 20 °C and transferred for central storage in Oslo University Hospital (-76 °C) within four weeks. Each sample was thawed and separated into two aliquots and subsequently sent on dry ice and batch analysed at Allergy department of the University of Athens.

Virus analyses were performed using real- time PCR at the Department of Allergy, University of Athens and is described in detail in Journal of Infectious Diseases (16). Respiratory syncytial virus, HRV as well as HRV subtypes A-B and C were analysed together with other common respiratory viruses (Influenza A, including H5N1, H1N1 virus, Influenza B, RSV A/B, MPV, AdV (B/C/E and some of A/D/F), CoV 229E/NL63/OC43, HRV A/B/C, Human Bocavirus 1/2/3/4 and Parainfluenza virus (PIV 1/2/3/4)).

High viral genomic load was identified using data-driven cluster analyses restricted to five clusters, and were subsequently classified as the high versus all other concentration clusters per virus (16). Details are outlined elsewhere (16).

3.2.3 Allergic sensitisation; s-IgE analyses

Blood samples were obtained by venous or capillary puncture. Serum was frozen at the local hospital, stored at Oslo University Hospital at a temperature of -76°C and analysed for s-IgE in one batch at Fürst Medical Laboratory, using ImmunoCAP by Phadia AB, Uppsala, Sweden. We first screened for allergic sensitisation with Phadiatop Infant® by Thermo Fisher Scientific, proceeding to s-IgE analyses to hen's egg white, cow's milk, peanut, cat, dog, birch, timothy and house dust mite in all samples with Phadiotop Infant level of at least 0.15 kU/l (n=89). All analyses were specified down to 0.10 kU/l. Allergic sensitisation was defined as s-IgE of at least 0.35 kU/l to at least one allergen, whereas low sensitisation was defined as s-IgE level of 0.10-0.34 kU/l.

3.2.4 Salivary cortisol sampling and analysis

Salivary cortisol sampling was performed the first morning after hospital admission, as soon as possible after the child's awakening (after 6:00 a.m.) and before the child's first meal, using two sorbettes (hydrocellulose, Salimetrics Europe Ltd, Suffolk, UK) as previously described in detail (105). The sorbettes were thereafter frozen at −86°C until transfer to Karolinska Institutet, Stockholm, for analysis in one batch by radioimmunoassay (100).

3.2.5 Supportive care

The use of supportive care in terms of nasogastric-tube feeding, oxygen therapy and ventilatory support by continuous positive airways pressure (CPAP) or intubation were initiated by the attending physician and recorded for the study on a daily basis by nurses. To ensure their completeness, all patient records were also reviewed by study members after the hospital stay.

3.2.6 Follow-up investigation at two years

The investigation was performed by specially trained physicians and nurses. In the physician-led structured interview, the parent was asked: Have episodes of respiratory distress/ wheezing occurred?; yes/no. If yes, specify number of episodes during the first and second year of life.

In addition to the structured interview, the child underwent a clinical examination, measurements of trans epidermal water loss, blood tests, skin prick-test and saliva cortisol sampling. None of these results are a part of/ accounted for in the present theses. For this thesis, only the information about the total number of episodes of wheeze in the bronchiolitis group was used, registered both at inclusion in the study (≤one previous obstructive episode was allowed for inclusion) and at two years of age.

3.3 Outcomes, exposures and covariates

An overview of outcomes, exposures and covariates are provided in **Table 3**.

Supportive care was defined as use of nasogastric-tube feeding, oxygen therapy and ventilatory support and used in analyses as a proxy for disease severity and to assess treatment response **(Table 3)**.

Length of hospital stay was defined as the time from the first study inhalation until discharge from hospital, as recorded in the medical record for each patient and used in analyses as a proxy for disease severity and to assess treatment response **(Table 3)**.

Allergic sensitisation was defined as s-IgE ≥0.35 kU/l, low allergic sensitisation was defined as s-IgE 0.1- 0.34 kU/l. Both Allergic sensitisation and low allergic sensitisation were used as dichotomous variables.

Recurrent wheeze was used as a proxy for early asthma, defined as parental reports of at least three separate episodes of bronchial obstruction at any time, including the acute bronchiolitis at study enrolment **(Table 3)**.

Parental **VAS** (10 being most severe) **clinical score** (0-10), 10 being most severe, used by physicians.

Virus; RSV, HRV, and HRV subtypes A-B and C and viral genomic load of RSV and HRV.

Salivary morning cortisol (mmol/l) was sampled first morning after hospital admission and used as a continuous variable in analyses.

Table 3 *Aims, outcomes, exposures and covariates in the thesis*

Aims	Outcomes	Exposures	Covariates
To identify risk factors for receiving supportive care for acute, moderate to severe infant bronchiolitis.	- Nasogastric tube feeding - Oxygen therapy - Ventilatory support	- Respiratory rate - SpO_2 - Age - Virus (HRV, HRV) - Allergic sensitisation - Eczema	- Sex - Gestational age - Caesarean section delivery - Parental allergic disease - Smoking at home - Cat or dog at home
To determine if clinical score or parental assessment using a visual analogue scale of acute infant bronchiolitis at the time of hospitalisation predicts the short-term prognosis.	- Length of hospital stay - Nasogastric tube feeding - Oxygen therapy - Ventilatory support	- Clinical score - Parental VAS score	- Sex - Age
To determine if severity of acute bronchiolitis, defined by length of hospital stay, receiving supportive care, clinical score or parental visual analogue scale is associated with early asthma development.	- Recurrent wheeze at two years of age as a proxy for early asthma	- Length of hospital stay - Nasogastric tube feeding - Oxygen therapy - Ventilatory support - Clinical score - Parental VAS score	- Age - Sex
To determine prevalence of early allergic sensitization and the role of allergic sensitisation, type or load of viruses or salivary cortisol during acute bronchiolitis in infancy for asthma development.	- Allergic sensitisation - Recurrent wheeze at two years of age as a proxy for early asthma	- RSV - RSV genomic load - HRV - HRV subtypes A-B and C - HRV genomic load - Allergic sensitisation - Salivary morning cortisol level	- Sex - Age - Gestational age - Caesarean section delivery - Birth weight - Eczema - Parental atopy - Exposure time

SpO_2 - Peripheral capillary oxygen saturation, HRV - Human Rhinovirus, RSV - Respiratory Syncytial Virus, VAS - Visual Analogue Scale

3.4 Statistical methods

Continuous data were analysed using Students t-test and presented as mean values with SD, minimum and maximum or 95 % CI, when appropriate. Categorical data, analysed by Pearson chi-square test, are given as numbers and percentages.

To identify risk factors for receiving supportive care for acute moderate to severe infant bronchiolitis we also applied bivariate and multivariate logistic regression analyses.

Data on LOS had a non-normal distribution and comparisons between those receiving inhaled racemic adrenaline versus inhaled saline and inhalation strategies on- demand versus at fixed-schedules were assessed with the use of robust, two sample t-test and Huber's M-estimator, with 95% confidence intervals.

The parental VAS scores were categorised into quartiles and use of supportive care in upper versus lowest quartile were compared by Pearson chi-square. The associations between severity assessments by parental VAS scores and clinical score and use of supportive care were assessed by bivariate logistic regression analyses. Multivariate logistic regression analyses with Hosmer stepdown procedure were applied to develop an algorithm for predicting use of supportive care, including predictors with a significance level of $p<0.25$ in the bivariate analyses.

The diagnostic accuracy of parental assessments and the prediction algorithm were evaluated by receiver operating characteristic (ROC) analyses and optimal cut-off and the sensitivity, specificity, positive and negative likelihood ratios were calculated.

The association between the different scores and LOS (hours) was analysed by regression analyses, where the length of stay was normalized by a power transformation; $hours^{0.3}$. The optimal exponent was estimated to be 0.3 by a Box-Cox analysis.

Logistic regression analyses were performed to study the association between the recurrent wheeze and RSV, HRV, HRV subtypes A-B and C, viral genomic load of RSV and HRV, allergic sensitisation to any allergen, to any food allergen or any inhalant allergen, or to single allergens, as well as salivary morning cortisol level. The actual counts were used as outcome in a truncated Poisson regression. The trunctation is due to all children included in the study had experienced at least one incidence of hospitalization due to wheezing. The Poisson model showed signs of overdispersion and was replaced by a negative binomial model. Both models were adjusted for age, sex and parental atopy and exposure time, given by the time from birth to the time of the two-year follow-up used as an offset. Interactions between these explanatory variables were also tested.

The significance level was set to 0.05 (5%). Analyses were performed using IBM SPSS version 22.0 and 25.0 (IBM, New York, USA.), SAS version 9.4 (SAS Institute Inc., North Carolina, USA) and R 3.3.0 and 3.6.0 (The R Foundation, Vienna, Austria).

3.5 Ethical considerations

The study was approved by the Regional Committees for Medical and Health Research Ethics and by the Norwegian Medicine Agency. The Bronchiolitis ALL South-East Norway study was registered at Clinicaltrial.gov number, NCT00817466, EudraCT number, 2009-012667-34 and was registered in the Norwegian Biobank Registry. The study was audited by the Norwegian Medicines Agency in 2011. Written informed consent was obtained from both parents/ guardians if possible, otherwise by one of the parents of each child before study enrolment. The study was conducted in line with Good Clinical Practice. All patients were insured through the Drug Liability Association of Norway.

3.6 Study population

The 404 infants included at hospital admission with acute bronchiolitis, (59.4% boys) had a mean age of 126 days (4.2 months) at enrolment, while the control group of 240 children, (55.8% boys) had a mean age of 197 days (6.6 months) at enrolment. **Figure 3** presents an overview of the study populations in the different papers.

The bronchiolitis group ***(paper 1)*** included the 404 infants hospitalized with acute bronchiolitis, and the baseline characteristics are shown in **Table 4**.

Paper 2 included infants with completed parental three-item VAS at study enrolment (n= 267 (66.1%)). Apart from higher maternal educational attainment (p=0.026) and a higher percentage of Caucasian mothers (p= 0.048) among the included infants, they were largely similar to infants who were not included due to missing parental assessments **(Table 4)**.

Paper 3 included all infants with available serum for IgE-analyses; 368 out of 404 (91.1%) infants in the bronchiolitis group (mean age 4.2 months) and 224 out of 240 (93.3%) infants in the control group (mean age 6.6 months), with an overall mean age of 5.1 months.

Paper 4 included those children in the bronchiolitis group attending 2-years follow-up (n= 294 (73%)). The mean age of these children was 24.6 (95% CI 24.3- 25) months at follow-up. 161 (61.6%) of the 294 patients were boys **(Table 4)**.

Table 4 *Background characteristics are shown for the 404 infants hospitalised with acute bronchiolitis, with comparison between the study population included and not included (grey shading) in papers # 2 (Parental VAS) and #4 (2-year follow-up), respectively.*

	Bronchiolitis group (N-404)	**Parental VAS at inclusion** (N-267)	**No parental VAS At inclusion** (N-137)	*p*	**Attending two-years follow-up** n= 294 (72.8)	**No two- years follow-up** n= 110 (27.2)	*p*
At birth Male sex n (%)	240/ 404 (59.4)	161/ 267 (60.3)	79/137 (57.7)	*0.61*	181/294 (61.6)	59/110 (53.6)	*0.15*
Gestational age (GA) weeks (SD)	38.8 (2.4)	38.6 (3.5)	38.7 (2.29)	*0.76*	38.6 (3.3)	38.8 (2.8)	*0.54*
Born at GA<37 weeks, n (%)	60/404 (14.9)	42/267 (15.7)	18 /137 (13.1)	*0.49*	46/294 (15.6)	14/110 (12.7)	*0.46*
Birth weight, grams (SD)	3440 (612)	34444 (662)	3397 (569)	*0.51*	3424 (641)	3450 (615)	*0.74*
At enrolment							
Age, days (range)	127.3 (7, 364)	127.9 (7, 364)	123.9 (9,362)	*0.66*	124.6 (7,363)	134.6 (10, 364)	*0.31*
Weight, grams (SD)	6510 (1874)	6558 (2925, 11655)	6417 (3230, 10750)	*0.48*	6442 (1847)	6692 (1942)	*0.23*
Eczema n (%)	40/ 374 (10.7)	29/ 253 (11.5)	11/121 (9.1)	*0.49*	29/271 (10.7)	11/103 (10.7)	*1*
One previous episode of wheeze	98/ 362 (27.1)	69 /245 (28.2)	29/ 117 (24.8)	*0.50*	69/ 260 (26.5)	29/ 102 (28.4)	*0.72*
Length of hospital stay, hours (SD)	80.2 (67.3)	82.0 (67.6)	76.9 (66.7)	*0.47*	80.3 (66.0)	80.0 (70.9)	*0.97*
Receiving supportive care, n (%)	204/404 (50.5)	133/267 (49.8)	71/ 137 (51.8)	*0.70*	152/294 (51.7)	52/110 (47.3)	*0.43*
Virus detected during acute bronchiolitis							
RSV, n (%)	300/363 (82.6)	202/ 244 (82.8)	98/ 119 (82.4)	*0.92*	219/266 (82.3)	81/97 (83.5)	*0.79*
HRV, n (%)	122/ 363 (33.6)	87 /244 (35.7)	35/ 119 (29.4)	*0.24*	93/266 (35.0)	29/97 (29.9)	*0.37*
HRV A or B, n (%)	35/ 363 (9.6)	28/ 244 (11.5)	7/ 119 (5.9)	*0.09*	28/266 (10.5)	7/97 (7.2)	*0.34*
HRV C, n (%)	87/ 363 (24.0)	59/ 244 (24.2)	28/ 119 (23.5)	*0.89*	65/266 (24.4)	22/97 (22.7)	*0.73*
RSV, high genomic load, n (%)	200/ 363 (55.1)	131/ 244 (53.7)	69/ 119 (58.0)	*0.44*	145/266 (54.5)	55/97 (56.7)	*0.71*
HRV, high genomic load, n (%)	23/ 363 (6.3)	16/ 144 (6.6)	7/ 119 (5.9)	*0.80*	16/266 (6.0)	7/97 (7.2)	*0.68*
More than 1 virus, n (%)	224/ 363 (61.7)	158/ 244 (64.8)	86/ 139 (35.2)	*0.09*	170/ 266 (63.9)	54/97 (55.7)	*0.15*
Allergic sensitisation, IgE ≥ 0.35							
Any sensitisation, n (%)	31/368 (8.4)	18/ 249 (7.2)	13/119 (10.9)	*0.23*	22/271 (8.1)	9/97 (9.3)	*0.72*
Any food sensitisation, n (%)	28/ 368 (7.6)	16/ 249 (6.4)	12/ 119 (10.1)	*0.22*	20/271 (7.4)	8/97 (8.2)	*0.78*
Any inhalant sensitisation, n (%)	7/ 364 (1.9)	5/ 247 (2.0)	2/ 117 (1.7)	*0.84*	5/267 (1.9)	2/97 (2.1)	*0.91*
Egg sensitisation, n (%)	12/ 368 (3.3)	8/249 (3.2)	4/ 115 (3.4)	*0.94*	8/271 (3.0)	4/97 (4.1)	*0.58*
Cow's milk sensitisation, n (%)	17/ 368 (4.6)	8/ 249 (3.2)	9/ 119 (7.6)	*0.06*	13/271 (4.8)	4/97 (4.1)	*0.79*
Peanut sensitisation, n (%)	4/ 368 (1.1)	3/ 249 (1.2)	1/ 119 (0.8)	*0.75*	3/271 (1.1)	1/97 (1.0)	*0.95*
Polysensitisation, n (%)	7/ 365 (1.9)	4/247 (1.6)	3/ 118 (2.5)	*0.55*	6/268 (2.2)	1/97 (1.0)	*0.46*

	Bronchiolitis group (N-404)	**Parental VAS at inclusion** (N-267)	**No parental VAS At inclusion** (N-137)	*p*	**Attending two-years follow-up** n= 294 (72.8)	**No two- years follow-up** n= 110 (27.2)	*p*
Cortisol geometric mean, mmol/l (95% CI)	48.8 (36.2)	NA	NA	*NA*	42.0 (32.9, 53.7)	35.2 (30.9, 10.0)	*NA*
Parental education							
Maternal Education[a] (SD)	3.9 (1.0)	3.9 (0.9)	3.7 (1.2)	***0.03***	3.99 (0.98)	3.47 (0.98)	***<0.001***
Paternal Education[a] (SD)	3.8 (1.0)	3.8 (0.9)	3.7 (1.1)	*0.52*	3.85 (1.0)	3.57 (0.92)	***0.019***
Parental allergic diseases							
Any n (%)	179/366 (48.9)	121/248 (48.7)	58/ 118 (49.2)	*0.95*	128/258 (49.6)	46/99 (46.5)	*0.59*
Maternal Asthma, n (%)	48/ 325 (14.8)	37/219 (16.9)	11/106 (10.4)	*0.12*	36/ 235 (15.3)	12/90 (13.3)	*0.65*
Paternal Asthma, n (%)	43/ 325 (13.2)	27/219 (12.3)	16/106 (15.1)	*0.49*	31/235 (13.2)	12/90 (13.3)	*0.97*
Maternal Rhinoconjungtivitis, n (%)	62/356 (17.4)	42/244 (17.4)	20/112 (17.9)	*0.88*	42/258 (16.3)	20/98 (20.4)	*0.36*
Paternal Rhinoconjungtivitis, n (%)	70/ 356 (19.7)	48/244 (19.7)	22/112 (19.6)	*1.00*	56/258 (21.7)	14/98 (14.3)	*0.12*
Maternal eczema, n (%)	40/ 361 (11.1)	30/200 (15.0)	14/ 92 (15.2)	*0.96*	31/263 (11.8)	9/98 (9.2)	*0.48*
Paternal eczema, n (%)	29/ 361 (8.0)	19/ 200 (9.5)	15/ 92 (16.3)	*0.09*	25/263 (9.5)	4/ 98 (4.1)	*0.092*
Environment Smoking at home n (%)	58/ 341 (17.0)	34/233 (14.6)	24/108 (22.2)	*0.08*	38/253 (15.0)	20/88 (22.7)	*0.097*

Comparing those with parental visual analogue scales *(VAS) and those without and those attending two- years follow-up and who did not. [a] Education was categorised from 1 (no school completed) to 5 (higher education, more than three years)*

4 Results

4.1 Risk factors for receiving supportive care for acute moderate to severe infant bronchiolitis. (Papers #1, 2)

Overall, 205/404 (50.7%) of the infants received supportive care during the hospital stay, with oxygen therapy in 166/404 (41.1%), nasogastric tube feeding in 116/404 (28.7%) and ventilatory support by CPAP in 30/404 (7.4%). None of the infants were intubated.

As shown in **Table 5** to **Table 8** and in **Table 9** preterm delivery and Caesarean section delivery were both significantly more common among infants who received any supportive care, and was significantly associated with receiving supportive care, not found for each of the treatment modalities.

Both age and weight at hospitalization were inversely/negatively and significantly associated with receiving supportive care.

Although infants receiving ventilatory support more often had fathers with reported rhinoconjunctivitis, parental reported allergic diseases were not associated with receiving supportive care or not.

We found no significant associations between second-hand smoking or having cats and/or dogs at home and receiving supportive care during acute infant bronchiolitis.

Table 5 *Characteristics of infants with acute bronchiolitis who did and did not receive supportive care*

		Supportive care n= (205)	No supportive care n= (199)	p
At birth	Male sex n (%)	110/ 205 (53,7)	130/ 199 (65.3)	**0.017**
	Gestational age (GA) weeks (95% CI)	38.4 (38,38.4)	39.2 (38.9, 39.5)	**0.001**
	Born at GA<37 weeks, n (%)	32/185 (17.3)	16/ 162 (9.0)	**0.02**
	Caesarean section delivery, n (%)	51/185 (27.6)	33/ 183 (18)	**0.03**
	Birth weight, grams (95% CI)	3360 (3256, 3464)	3478 (3399, 3557)	0.08
Medical history				
	One previous episode of wheeze (%)	47/138 (25.4)	51/126 (28.8)	0.47
	Eczema n (%)	12/189 (6.3)	28/185 (15.1)	**0.006**
	Allergy n (%)	4/190 (2.1)	3/183 (1.6)	0.74
At enrolment				
	Age, days (95% CI)	117 (104, 130)	138 (127, 149)	**0.02**
	Age ≤3 months	104/205 (50.7)	73/199 (36.7)	**0.004**
	Age ≤6 months	159/205 (77.6)	140/199 (70.4)	0.1
	Weight, grams (95% CI)	6099 (5840, 6350)	6933 (6684, 7182)	**<0.001**
	Respiratory rate (95% CI)	55 (54, 56)	52 (50, 54)	**0.014**
	Respiratory rate ≥ 60/min	88/195 (45.1)	63/ 192 (32.8)	**0.013**
	Respiratory rate ≥ 50/min	135/195 (69.2)	116/192 (60.4)	0.07
	SpO_2 % [a] (95% CI)	95 (94.6, 95.4)	97 (96.7, 97.3)	**<0.001**
	SpO_2 <90%[a], n (%)	15/199 (7.5)	3/190 (1.6)	**0.005**
	SpO_2 <92%[a], n (%)	25/199 (12.6)	5/190 (2.6)	**<0.001**
	SpO_2 <94% [a], n (%)	51/199 (25.6)	15/175 (7.9)	**<0.001**
	SpO_2 <96% [a], n (%)	91/199 (45.7)	47/143 (24.7)	**<0.001**
	SpO_2 <98% [a], n (%)	149/199 (74.9)	105/190 (55.3)	**<0.001**
	Heart rate beats per minute (95% CI)	156 (153, 159)	151 (148, 154)	**0.01**
	Clinical Score (95% CI)	5 (4.9, 5.1)	4.8 (4.7, 4.9)	**0.024**
	Cortisol, mmol/l (95% CI)	54.2 (46.7, 61.7)	42.9 (35.8, 50)	**0.014**
Virus detected during acute bronchiolitis				
	RSV, n (%)	158/187 (84.5)	142/176 (80.7)	0.34
	HRV, n (%)	60/ 187 (32.1)	62/176 (35.2)	0.53
	HRV A or B, n (%)	18/187 (9.6)	17/ 176 (9.7)	0.99
	HRV C, n (%)	42/187 (22.5)	45/176 (25.6)	0.49
	RSV, high genomic load, n (%)	116/ 187 (62.0)	84/ 176 (47.7)	**0.006**
	HRV, high genomic load, n (%)	10/187 (5.3)	13/ 176 (7.4)	0.43
	More than 1 virus, n (%)	110/187 (58.8)	113/ 176 (64.2)	0.29
Allergic sensitisation, IgE ≥ 0.35 kU/l				
	Any sensitisation, n (%)	16/184 (8.7)	15/180 (8.3)	0.90
	Any food sensitisation, n (%)	15/184 (8.2)	13/180 (7.2)	0.74
	Any inhalant sensitisation, n (%)	3/184 (1.6)	4/180 (2.2)	0.68
	Polysensitisation, n (%)	5/184 (2.7)	4/180 (2.2)	0.77
Parental VAS item				
	Activity (95% CI)	4.4 (3.9, 4.9)	6 (5.6, 6.5)	**<0.001**
	Eating (95% CI)	4 (3.5, 4.6)	5.8 (5.3, 6.3)	**<0.001**
	Illness (95% CI)	3.6 (3.3, 3.9)	4.7 (4.4, 5.0)	**<0.001**
Inhalation strategies				
	Adrenaline inhalation therapy, n (%)	99/205 (48.3)	104/199 (52.3)	0.43
	Inhalations given on demand, n (%)	92/ 205 (44.9)	108/199 (54.3	0.06
	Length of hospital stay, hours (95% CI)	114 (104, 124)	45 (40,50)	**<0.001**
Parental education[b]				
	Maternal Education[a] (95% CI)	3.9 (3.8, 4.0)	3.8 (3.7, 4.0)	0.28
	Paternal Education[a] (95% CI)	3.9 (3.8, 4.1)	3.7 (3.6, 3.9)	0.1
Parental allergic diseases				
	Any n (%)	89/184 (48.5)	82/180 (45.6)	0.59
	Maternal Asthma, n (%)	23/168 (13.7)	25/ 157 (15.9)	0.57
	Paternal Asthma, n (%)	20/168 (11.9)	23/157 (14.6)	0.47
	Maternal Rhinoconjuncivitis, n (%)	32/ 182 (17.6)	30/ 174 (17.2)	0.93
	Paternal Rhinoconjuncivitis, n (%)	41/182 (22.5)	29/ 174 (16.7)	0.16
	Maternal eczema, n (%)	19/183 (10.4)	21/178 (11.8%)	0.67
	Paternal eczema, n (%)	12/183 (6.6)	17/ 178 (9.6%)	0.30
Environment	Smoking at home n (%)	28/172 (16.3)	30/169 (17.8)	0.72
	Cat n (%)	34/168 (20.3)	28/ 169 (16.6)	0.39
	Dog n (%)	20/170 (11.8)	29/ 167 (17.4)	0.15

[a] SpO2 denotes peripheral capillary oxygen saturation by pulse oximetry. [b] Education was categorised from 1 (no school completed) to 5.

Table 6 *Characteristics of infants with acute bronchiolitis who did and did not receive oxygen therapy*

		Oxygen therapy n= (166)	No Oxygen therapy n= (215)	p
At birth	Male sex n (%)	90/ 166 (65.3)	134/ 215 (62.3)	0.11
	Gestational age (GA) weeks (95% CI)	38.5 (38.1, 39.0)	39.2 (38.7, 39.3)	0.07
	Born at GA<37 weeks, n (%)	23/149 (15.4)	22/ 191 (11.5)	0.29
	Caesarean section delivery, n (%)	38/151 (25.2)	43/ 196 (21.9)	0.48
	Birth weight, grams (95% CI)	3412 (3299, 3525)	3438 (3356, 320)	0.71
Medical history				
	One previous episode of wheeze (%)	36/148 (24.3)	56/192 (29.2)	0.32
	Eczema n (%)	6/152 (3.9)	33/199 (16.6)	**<0.001**
	Allergy n (%)	3/153 (2.0)	4/197 (2.0)	0.96
At enrolment				
	Age, days (95% CI)	110 (96, 124)	143 (132, 154)	**<0.001**
	Age ≤3 months	92/166 (55.4)	73/215 (34.0)	**<0.001**
	Age ≤6 months	135/166 (81.3)	145/215 (67.4)	**0.002**
	Weight, grams (95% CI)	5996 (5705, 6287)	6937 (6701, 7173)	**<0.001**
	Respiratory rate (95% CI)	55 (54, 56)	52 (50, 54)	**0.013**
	Respiratory rate ≥ 60/min	74/158 (46.8)	68/ 207 (32.9)	**0.007**
	Respiratory rate ≥ 50/min	110/159 (69.6)	127/207 (61.4)	0.10
	SpO_2 %[a] (95% CI)	95 (94, 96)	97 (96.7, 97.3)	**<0.001**
	SpO_2 <90%[a], n (%)	15/162 (9.3)	2/203 (1.0)	**<0.001**
	SpO_2 <92%[a], n (%)	24/162 (14.8)	5/200 (2.4)	**<0.001**
	SpO_2 <94%[a], n (%)	48/162 (29.6)	17/205 (8.3)	**<0.001**
	SpO_2 <96%[a], n (%)	80/162 (49.4)	51/154 (24.9)	**<0.001**
	SpO_2 <98%[a], n (%)	125/162 (77.2)	115/205 (56.1)	**<0.001**
	Pulse, beats per minute (95% CI)	155 (155, 158)	151 (149, 155)	0.07
	Clinical Score (95% CI)	5.1 (4.9, 5.3)	4.8 (4.7, 4.9)	**0.006**
	Cortisol, mmol/l (95% CI)	51.7 (43.8, 59.6)	45.2 (37.9, 52.5)	0.15
Virus detected during acute bronchiolitis				
	RSV, n (%)	126/151 (83.4)	156/191 (81.7)	0.67
	HRV, n (%)	46/ 151 (30.5)	69/191 (36.1)	0.27
	HRV A or B, n (%)	13/151 (8.6)	21/ 191 (11.0)	0.46
	HRV C, n (%)	33/151 (21.9)	48/191 (25.1)	0.48
	RSV, high genomic load, n (%)	95/ 151 (62.9)	91/ 191 (47.6)	**0.005**
	HRV, high genomic load, n (%)	10/151 (6.6)	12/ 191 (6.3)	0.90
	More than 1 virus, n (%)	88/151 (58.3)	122/ 191 (63.9)	0.29
Allergic sensitisation, IgE ≥ 0.35 kU/l				
	Any sensitisation, n (%)	12/152 (7.9)	17/190 (8.9)	0.73
	Any food sensitisation, n (%)	11/152 (7.2)	15/190 (7.9)	0.82
	Any inhalant sensitisation, n (%)	2/152 (1.3)	5/190 (2.6)	0.39
	Polysensitisation, n (%)	4/153 (2.6)	5/190 (2.6)	0.99
Parental VAS item				
	Activity (95% CI)	4.4 (3.9, 4.9)	5.8 (5.4, 6.2)	**<0.001**
	Eating (95% CI)	4.3 (3.7, 4.9)	5.3 (4.8, 5.8)	**0.015**
	Illness (95% CI)	3.5 (3.1, 3.9)	4.5 (4.2, 4.8)	**<0.001**
Inhalation strategies				
	Adrenaline inhalation therapy, n (%)	83/166 (50.0)	106/215 (49.3)	0.89
	Inhalations given on demand, n (%)	72/ 166 (43.4)	116/215 (54.0)	**0.04**
	Length of hospital stay, hours (95% CI)	122 (111, 133)	46 (41, 51)	**<0.001**
Parental education[b]				
	Maternal Education[a] (95% CI)	3.9 (3.7, 4.1)	3.8 (3.7, 4.0)	0.56
	Paternal Education[a] (95% CI)	3.8 (3.6, 4.0)	3.7 (3.6, 3.8)	0.22
Parental allergic diseases				
	Any n (%)	76/150 (50.7)	90/192 (49.9)	0.49
	Maternal Asthma, n (%)	20/138 (14.5)	28/ 171 (16.4)	0.65
	Paternal Asthma, n (%)	18/138 (13.0)	24/171 (14.0)	0.80
	Maternal Rhinoconjuncivitis, n (%)	28/ 148 (18.9)	33/ 186 (17.7)	0.78
	Paternal Rhinoconjuncivitis, n (%)	35/148 (23.6)	31/ 186 (16.7)	0.11
	Maternal eczema, n (%)	16/149 (10.7)	23/190 (12.1)	0.70
	Paternal eczema, n (%)	10/149 (6.7)	19/ 190 (10.0)	0.28
Environment	Smoking at home n (%)	23/143 (16.1)	29/176 (16.5)	0.93
	Cat n (%)	25/140 (17.9)	33/ 175 (18.9)	0.82
	Dog n (%)	15/141 (10.6)	32/ 174 (18.4)	0.06

[a] SpO2 denotes peripheral capillary oxygen saturation by pulse oximetry. [b] Education was categorised from 1 (no school completed) to 5.

Table 7 *Characteristics of infants with acute bronchiolitis who did and did not receive nasogastric tube feeding*

		Nasogastric tube feeding n= (116)	No nasogastric tube feeding n= (284)	p
At birth	Male sex n (%)	62/116 (53.4)	174/284 (61.3)	0.15
	Gestational age (GA) weeks (95% CI)	38.4 (37.9, 38.9)	38.9 (38.6, 39.2)	0.07
	Born at GA<37 weeks, n (%)	17/105 (16.2)	31/253 (12.2)	0.31
	Caesarean section delivery, n (%)	27/103 (26.2)	56/261 (21.5)	0.33
	Birth weight, grams (95% CI)	3348 (3219, 3486)	3443 (3359, 3517)	0.21
Medical history				
	One previous episode of wheeze (%)	24/107 (22.4)	73/252 (29)	0.20
	Eczema n (%)	9/108 (8.3)	31/262 (11.8)	0.32
	Allergy n (%)	2/108 (1.9)	5/261 (1.9)	0.97
At enrolment				
	Age, days (95% CI)	116 (99, 133)	132 (122, 142)	0.10
	Age ≤3 months	63/116 (54.3)	112/284 (39.4)	**0.007**
	Age ≤6 months	86/116 (74.1)	210/284 (73.9)	0.97
	Weight, grams (95% CI)	5985 (5641, 6329)	6714 (6501, 6927)	**<0.001**
	Respiratory rate (95% CI)	56 (54,58)	53 (51, 54)	**0.013**
	Respiratory rate ≥ 60/min	51/108 (47.1)	98/275 (35.6)	**0.04**
	Respiratory rate ≥ 50/min	77/108 (71.3)	172/275 (62.5)	**0.11**
	SpO_2 %[a] (95% CI)	95 (94, 96)	96 (95.5, 96.5)	**<0.001**
	SpO_2 <90%[a], n (%)	9/102 (8,1)	9/274 (3.3)	**0.04**
	SpO_2 <92%[a], n (%)	12/111 (10.8)	18/274 (6.6)	0.16
	SpO_2 <94%[a], n (%)	27/111 (24.3)	39/174 (14.2)	**0.02**
	SpO_2 <96%[a], n (%)	54/111 (48.6)	82/274 (29.9)	**<0.001**
	SpO_2 <98%[a], n (%)	82/111 (73.9)	168/274 (61.3)	**0.02**
	Pulse, beats per minute (95% CI)	157 (154, 160)	152 (150, 155)	**0.03**
	Clinical Score (95% CI)	5.2 (5.0, 5.4)	4.8 (4.7, 4.9)	**0.001**
	Cortisol, mmol/l (95% CI)	58 (47, 69)	45 (39, 51)	**0.03**
Virus detected during acute bronchiolitis				
	RSV, n (%)	89/106 (84.0)	208/ 253 (82.2)	0.69
	HRV, n (%)	38/ 106 (35.8)	83/253 (32.8)	0.58
	HRV A or B, n (%)	11/106 (10.4)	23/253 (9.1)	0.7
	HRV C, n (%)	27/106 (25.5)	60/253 (23.7)	0.72
	RSV, high genomic load, n (%)	65/106 (61.3)	134/253 (53.0)	0.15
	HRV, high genomic load, n (%)	6/106 (5.7)	17/253 (6.7)	0.71
	More than 1 virus, n (%)	64/106 (60.4)	156/253 (61.7)	0.82
Allergic sensitisation, IgE ≥ 0.35 kU/l				
	Any sensitisation, n (%)	9/101 (8.9)	22/259 (8.5)	0.90
	Any food sensitisation, n (%)	9/101 (8.9)	19/259 (7.3)	0.62
	Any inhalant sensitisation, n (%)	1/100 (1.0)	6/259 (2.3)	0.41
	Polysensitisation, n (%)	2/102 (2.0)	7/259 (2.7)	0.68
Parental VAS item				
	Activity (95% CI)	3.9 (3.3, 4.5)	5.7 (5.3, 6.1)	**<0.001**
	Eating (95% CI)	3.1 (2.5, 3.8)	5.5 (5.0, 6.0)	**<0.001**
	Illness (95% CI)	3.3 (2.9, 3.7)	4.5 (4.2, 4.7)	**<0.001**
Inhalation strategies				
	Adrenaline inhalation therapy, n (%)	57/116 (49,1)	144/284 (50.7)	0.78
	Inhalations given on demand, n (%)	52/116 (44.8)	146/284 (51.4)	0.23
	Length of hospital stay, hours (95% CI)	125 (110, 140)	63 (57, 69)	**<0.001**
Parental education[b]				
	Maternal Education[a] (95% CI)	3.9 (3.7, 4.1)	3.8 (3.7, 3.9)	0.33
	Paternal Education[a] (95% CI)	3.9 (3.7, 4.1)	3.7 (3.6, 3.8)	0.2
Parental allergic diseases				
	Any n (%)	48/104 (46.2)	122/256 (47.7)	0.80
	Maternal Asthma, n (%)	11/97 (11.3)	37/224 (16.5)	0.23
	Paternal Asthma, n (%)	11/97 (11.3)	32/224 (14.3)	0.48
	Maternal Rhinoconjuncivitis, n (%)	18/102 (17.6)	44/250 (17.6)	0.99
	Paternal Rhinoconjuncivitis, n (%)	22/102 (21.6)	48/250 (19.2)	0.61
	Maternal eczema, n (%)	12/102 (11.8)	27/ 255 (10.6)	0.75
	Paternal eczema, n (%)	8/102 (7.8)	21/255 (8.2)	0.90
Environment	Smoking at home n (%)	14/95 (14.7)	44/242 (18.2)	0.45
	Cat n (%)	14/92 (15.2)	48/141 (19.9)	0.33
	Dog n (%)	9/92 (9.8)	40/ 241 (16.6)	0.12

[a] SpO2 denotes peripheral capillary oxygen saturation by pulse oximetry. [b] Education was categorised from 1 (no school completed) to 5.

Table 8 *Characteristics of infants with acute bronchiolitis who did and did not receive ventilatory support*

		Ventilatory support n= (30)	No Ventilatory support n= (374)	p
At birth	Male sex n (%)	20/30 (66.7)	220/374 (58.8)	0.40
	Gestational age (GA) weeks (95% CI)	38.6 (37.5, 39.2)	38.8 (38.4, 39.2)	0.71
	Born at GA<37 weeks, n (%)	5/28 (17.9)	43/335 (12.8)	0.45
	Caesarean section delivery, n (%)	7/30 (23.3)	77/338 (22.8)	0.95
	Birth weight, grams (95% CI)	3375 (3087, 3663)	3422 (3355, 3489)	0.71
Medical history				
	One previous episode of wheeze (%)	7/30 (23.3)	91/332 (27.4)	0.63
	Eczema n (%)	1/30 (3.3)	39/344 (11.3)	9.17
	Allergy n (%)	0/30 (0.0)	7/343 (2.0)	0.43
At enrolment				
	Age, days (95% CI)	65 (43, 87)	132 (123, 141)	**<0.001**
	Age ≤3 months	23/30 (76.7)	154/374 (41.2)	**<0.001**
	Age ≤6 months	29/30 (96.7)	270/374 (72.2)	**<0.001**
	Weight, grams (95% CI)	5013 (4497, 5529)	6630 (6442, 6818)	**<0.001**
	Respiratory rate (95% CI)	53.4 (49.1, 57.7)	53.5 (52.4, 54.7)	0.99
	Respiratory rate ≥ 60/min	9/27 (33.3)	142/360 (39.4)	0.53
	Respiratory rate ≥ 50/min	16/27 (59.3)	235/360 (65.3)	0.53
	SpO_2 %[a] (95% CI)	93 (91, 95)	96 (95.5, 96.5)	**0.003**
	SpO_2 <90%[a], n (%)	7/28 (25.0)	11/361 (3.0)	**<0.001**
	SpO_2 <92%[a], n (%)	8/28 (28.6)	22/361 (6.1)	**<0.001**
	SpO_2 <94%[a], n (%)	13/28 (46.6)	53/361 (14.7)	**<0.001**
	SpO_2 <96%[a],n (%)	17/28 (60.7)	121/361 (33.5)	**0.004**
	SpO_2 <98%[a], n (%)	23/28 (82.1)	231/361 (64.0)	**0.05**
	Pulse, beats per minute (95% CI)	157 (150, 164)	153 (151, 155)	0.28
	Clinical Score (95% CI)	5.4 (4.9, 5.9)	4.9 (4.8, 5.0)	**0.023**
	Cortisol, mmol/l (95% CI)	76.1 (49.8, 102.4)	47.4 (42.1, 52.8)	**0.02**
Virus detected during acute bronchiolitis				
	RSV, n (%)	25/27 (92.6)	275/336 (81.8)	0.16
	HRV, n (%)	9/27 (33.3)	113/336 (33.6)	0.98
	HRV A or B, n (%)	3/27 (11.1)	32/336 (9.5)	0.79
	HRV C, n (%)	6/27 (22.2)	81/336 (24.1)	0.83
	RSV, high genomic load, n (%)	21/27 (77.8)	179/336 (53.3)	**0.01**
	HRV, high genomic load, n (%)	1/27 (3.7)	22/336 (6.5)	0.56
	More than 1 virus, n (%)	17/27 (63.0)	206/336 (61.3)	0.87
Allergic sensitisation, IgE ≥ 0.35 kU/l				
	Any sensitisation, n (%)	2/27 (7.4)	29/337 (8.6)	0.83
	Any food sensitisation, n (%)	2/27 (7.4)	26/337 (7.7)	0.95
	Any inhalant sensitisation, n (%)	9/27 (0.0)	7/337 (2.1)	0.45
	Polysensitisation, n (%)	1/28 (3.6)	8/ 337 (2.4)	0.70
Parental VAS item				
	Activity (95% CI)	4.0 (2.8, 5.2)	5.3 (5.0, 5.6)	**0.035**
	Eating (95% CI)	3.4 (2.0, 4.8)	5.0 (4.6, 5.4)	**0.038**
	Illness (95% CI)	3.2 (2.3, 4.1)	4.2 (4.0, 4.4)	**0.021**
Inhalation strategies				
	Adrenaline inhalation therapy, n (%)	15/30 (50.0)	188/374 (50.3)	0.98
	Inhalations given on demand, n (%)	8/30 (26.7)	192/374 (51.3)	**0.009**
	Length of hospital stay, hours (95% CI)	203.2 (170.5, 235.9)	70.4 (64.9, 75.9)	**<0.001**
Parental education[b]				
	Maternal Education[a] (95% CI)	3.8 (3.5, 4.1)	3.9 (3.8, 4.0)	0.77
	Paternal Education[a] (95% CI)	3.9 (3.5, 4.3)	3.8 (3.7, 3.9)	0.60
Parental allergic diseases				
	Any n (%)	19/30 (63.3)	152/334 (45.5)	0.06
	Maternal Asthma, n (%)	4/29 (13.8)	44/296 (14.9)	0.88
	Paternal Asthma, n (%)	6/29 (20.7)	37/296 (12.5)	0.21
	Maternal Rhinoconjunctivitis, n (%)	5/30 (16.7)	57/326 (17.5)	0.91
	Paternal Rhinoconjunctivitis, n (%)	12/30 (40.0)	58/326 (17.8)	**0.003**
	Maternal eczema, n (%)	5/30 (16.7)	35/331 (10.6)	0.31
	Paternal eczema, n (%)	5/30 (16.7)	24/331 (7.3)	0.07
Environment	Smoking at home n (%)	3/30 (10.0)	55/311 (17.7)	0.29
	Cat n (%)	2/29 (6.9)	60/308 (19.5)	0.1
	Dog n (%)	2/29 (6.9)	47/308 (15.3)	0.22

[a] SpO2 denotes peripheral capillary oxygen saturation by pulse oximetry. [b] Education was categorised from 1 (no school completed) to 5.

Increased respiratory rate, especially ≥60 per minute, was significantly associated with receiving oxygen therapy and nasogastric tube feeding as well as receiving supportive care overall. We identified a significant inverse association between SpO_2 and receiving supportive care, with oxygen saturation <92% giving the highest OR **(Table 9)**.

Neither RSV nor HRV or multiple viruses were significantly associated with receiving supportive care, while high RSV genomic load was significantly associated with receiving supportive care, especially ventilatory support **(Table 9)**.

There were no significant differences in receiving supportive care between children treated with inhaled racemic adrenaline and those treated with inhaled saline. Oxygen therapy and ventilatory support were significantly less frequent among infants receiving inhalation therapy on demand compared to fixed schedule, 43.4% versus 54%, p= 0.04 and 26.7% versus 51.3%, p<0.001 **(Table 6 and table 8).**

Inhalation therapy on demand was associated with significantly less frequent use of oxygen therapy and ventilatory support **(Table 9)**. Infants receiving inhalation therapy on demand received significantly fewer inhalations compared to infants receiving inhalations at fixed schedules with a mean (95% CI) of 12.8 (10.9, 14.6) versus 19.5 (17.0, 22.9) (mean difference 6.7) inhalations, p<0.001.

Table 9 *Odds Ratios (OR), by bivariate analyses adjusted for age and gender, for receiving supportive care during acute bronchiolitis is shown by univariate analyses for background and clinical characteristics at hospitalisation.*

	Supportive care OR (95% CI)		**Oxygen therapy OR (95% CI)**		**Nasogastric tube feeding OR (95% CI)**		**Ventilatory support OR (95% CI)**	
At birth		*p*		*p*		*p*		*p*
Male sex	1.64 (1.10, 2.46)	***0.016***	1.40 (0.92, 2.13)	0.12	1.38 (0.89, 1.0)	0.15	0.69 (0.31, 1.54)	0.37
Gestational age (GA) weeks	0.84 (0.76, 0.92)	***<0.001***	0.89 (0.81, 0.97)	***0.01***	0.91 (0.83, 0.99)	***0.04***	0.89 (0.75, 1.05)	0.17
Born at GA<37 weeks	2.34 (1.22, 4.51)	***0.01***	1.63 (0.85, 3.13)	0.14	1.48 (0.77, 2.83)	0.24	2.25 (0.76, 6.65)	0.14
Caesarion section delivery	2.1 (1.2, 3.5)	***0.006***	1.53 (0.90, 2.60)	0.12	1.44 (0.83, 2.49)	0.19	1.74 (0.68, 4.48)	0.25
Birth weight, grams	1	***0.04***	1	0.39	1	0.11	1	0.21
At enrolment								
Age, days	1 (1,1)	***0.02***	1 (0.99, 1)	***<0.001***	1 (1,1	0.146	0.99 (0.98, 0.99)	***<0.001***
Age ≤3 months	1.76 (1.18, 2.62)	***0.006***	2.39 (1.58, 3.63)	***<0.001***	1.81 (1.17, 2.80)	***0.008***	4.79 (2.0, 11.47)	***<0.001***
Age ≤6 months	1.46 (0.93, 2.29)	0.10	2.10 (1.29, 3.41)	***0.003***	1.01 (0.62, 1.65)	0.98	11.13 (1.51, 83.52)	***0.018***
Weight, grams	1 (1,1)	***<0.001***	1 (1,1)	***0.002***	1	***<0.001***	1	***0.05***
Diagnosed with eczema	0.46 (0.22, 0.94)	***0.035***	0.27 (0.11, 0.68)	***0.005***	0.78 (0.45. 1.73)	0.54	0.67 (0.08, 5.49)	0.71
Clinical condition at enrolment								
Heart rate, beats per minute	1.01 (1.0, 1.02)	***0.03***	1.01 (1, 1.02)	0.16	1.01 (1, 1.03)	0.06	1,01 (0.98, 1.03)	0.57
Respiratory rate	1.02 (1.00, 1.04)	***0.02***	1.02 (1,1.04)	***0.02***	1.03 (1.0, 1.05)	***0.02***	1 (0.97, 1.04)	0.95
Respiratory rate ≥ 60/min	1.68 (1.11, 2.55)	***0.015***	1.8 (1.17, 2.78)	***0.008***	1.61 (1.02, 2.53)	***0.04***	0.81 (0.35, 1.90)	0.63
Respiratory rate ≥ 50/min	1.45 (0.95, 2.22)	0.09	1.43 (0.91, 2.24)	0.12	1.47 (0.9, 2.39)	0.12	0.78 (0.34, 1.78)	0.56
SpO_2 %	0.79 (0.72, 0.85)	***<0.001***	0.76 (0.70, 0.83)	***<0.001***	0.89 (0.83, 0.95)	***0.001***	0.84 (0.76, 0.92)	***<0.001***
SpO_2 <90%	5.01 (1.41, 17.82)	***0.013***	10.28 (2.26, 46.8)	***0.003***	2.50 (0.95, 6.53)	0.06	8.8 (2.83, 27.41)	***<0.001***
SpO_2 <92%	5.29 (1.96, 14.25)	***0.001***	7.1 (2.60, 19.42)	***<0.001***	1.68 (0.77, 3.63)	0.19	5.68 (2.12, 15.26)	***0.001***
SpO_2 <94%	4.18 (2.23, 7.83)	***<0.001***	5.0 (2.68, 9.28)	***<0.001***	1.94 (1.11, 3.38)	***0.02***	4.90 (2.12, 11.33)	***<0.001***
SpO_2 <96%	2.66 (1,72, 4.14)	***<0.001***	3.13 (1.98, 4.93)	***<0.001***	2.27 (1.43, 3.58)	***<0.001***	3.11 (1.38, 7.01)	***0.006***
SpO_2 <98%	2.59 (1.67, 4.02)	***<0.001***	2.89 (1.80, 4.62)	***<0.001***	1.85 (1.13, 2.02)	***0.014***	2.73 (1.0, 7.48)	***0.05***
Test results								
RSV, high genomic load	1.78 (1.17, 2.73)	***0.008***	1.88 (1.20, 2.93)	***0.006***	1.40 (0.88, 2.23)	0.16	3.01 (1.18, 8.08)	**0.02**
Cortisol, mmol/l (95% CI)	1.01 (1.0, 1.02)	***0.04***	1.01 (1.0, 1.01)		1.01 (1.0, 1.02)	0.05	1.02 (1.0, 1.03)	**0.04**
Severity assessment at enrolment								
Clinical Score	1.28 (1.04, 1.56)	***0.02***	1.36 (1.12, 1.68)	***0.004***	1.51 (1.21, 1.86)	***<0.001***	1.61 (1.15,2.26)	***0.005***
Parent VAS; Activity	1.26 (1.15, 1.39)	***<0.001***	0.22 (1.10, 1.35)	***<0.001***	1.30 (1.16, 1.45)	***<0.001***	1.23 (1.02, 1.49)	***0.03***
Parent VAS; Eating	1.23 (1.13, 1.34	***<0.001***	1.14 (1.05, 1.25)	***0.002***	1.32 (1.19, 1.46)	***<0.001***	1.28 (0.97, 0.99)	***0.006***
Parent VAS; Illness	1.36 (1.18, 1.56)	***<0.001***	1.33 (1.15, 1.54)	***<0.001***	1.40 (1.20, 1.63)	***<0.001***	1.35 (1.04, 1.75)	***0.03***
Inhalations given on demand	0.67 (0.45, 2.22)	***0.05***	0.64 (0.42, 0.97)	***0.034***	0.76 (0.49, 1.17)	0.22	0.36 (0.15, 0.84)	***0.02***
Length of hospital stay hours	1.03 (1.02, 1,04)	***<0.001***	1.03 (1.02, 1.04)	***<0.001***	1.02 (1.01, 1.02)	***<0.001***	1.02 (1.02, 1.03)	***<0.001***

The following variables: preterm birth (gestational age <37 weeks), Caesarion section delivery, heart rate, respiratory rate, SpO_2< 92%, RSV high genomic load and Inhalations given on demand, all being significantly associated in univariate analyses, were included in a multivariate logistic regression analysis, adjusted for male sex and age at inclusion.

In the multivariate logistic regression analyses Caesarion section delivery, SpO_2 < 92%, heart rate and age at hospitalisation were independent risk factors for receiving supportive care during hospital stay **(Table 10)**, with a Nagelkerke (R^2) of 0.12.

Table 10 *Odds Ratios (OR), by multivariate analyses, for receiving supportive care during acute bronchiolitis shown by multivariate analyses.*

	Supportive care OR (95% CI)	*p*
Age, days	1.00 (0.99, 1.00)	0.01
Male sex	1.40 (0.89, 2.20)	0.15
Caesarion section delivery	2.10 (1.20, 3.67)	0.01
Heart rate at inclusion, beats per minute	1.01 (1.00, 1.03)	0.03
SpO_2 <92% at inclusion	4.65 (1.52, 14.24)	0.007

Only variables with significant association (Odds Ratio (OR)) after applying Hosmer stepdown procedure in the multivariate regression analyses are presented, adjusted for age and gender.

4.2 Clinical score and parental assessment of acute infant bronchiolitis at the time of hospitalisation and short-term prognosis. (Papers #1, 2)

The mean (range) clinical score **(Table 2)** at hospitalisation with acute bronchiolitis was 4.9 (4-9), with a median of 5. The mean (range) of the parental VAS items: Activity, Eating and Illness was 5.22 (0-10), 4.87 (0;10) and 4.13 (0-9) with a median of 4.8, 4.8 and 4.2, respectively.

The clinical score

Length of Stay was found to be linearly dependent on the clinical score on a cubic scale.

The estimated relation (after back-transformation) is given by:

$$\text{LOS (hours)} = (3.18 + 0.14 * \text{clinical score})^3$$

$\beta_{\text{Clinical score}}$ (95%CI) 3.18 (2.59, 3.77), p= 0.007)

The clinical score at hospital admission was significantly associated with receiving supportive care, with nasogastric tube feeding having the highest OR (95% CI) of 1.51 (1.21, 1.86), corresponding to a 51% increase risk of receiving nasogastric tube feeding for each increase in point on the clinical score. The SpO_2 showed an OR of 0.77. Logically this implies that a one percent point increase in SpO2 leads to a 23% decrease in risk of receiving of supp care. This is equivalent to a 30% increase in risk of supportive care for a one percent-point decrease in SpO2. **(Table 11).**

The ability of the clinical score to predict any supportive care had a sensitivity and specificity of 59% and 45%, respectively **(Table 13)**.

The parental VAS- items

Length of Stay was found to be linearly dependent on VAS-activity and VAS- Illness on a cubic scale.

The estimated relation (after back-transformation) is given by:

$$LOS_{Activity} = (3.67 + 0.17 * VAS_{Activity})^3$$

$$LOS_{Illness} = (3.41 + 0.17 * VAS_{Illness})^3$$

Both VAS-items coefficients were found significant with p =0.005,

$\beta_{Activity}$ (95%CI) 3.67 (3.38, 3.96) and $\beta_{Illness}$ (95%CI) 3.41 (2.95, 3.87)

The equivalent model for the VAS item feeding was:

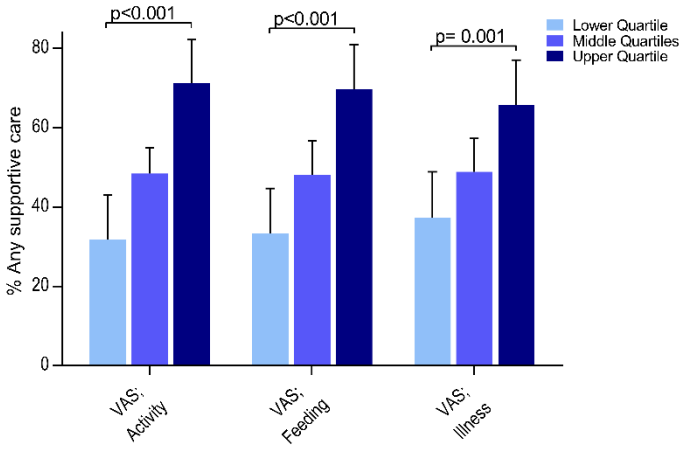
$$LOS_{Feeding} = (3.79 + 0.11 * VAS_{Feeding})^3$$

The coefficient for the $VAS_{feeding}$ was however not significant (p=0.066),

$\beta_{Feeding}$ (95%CI) 3.79 (3.52, 4.06).

Disease severity assessed by parental VAS was significantly associated with the use of any supportive care during hospital stay. Infants scored most severe (in upper quartile) in all three parental VAS items received supportive care significantly more often than infants scored in the lower quartiles **(Figure 6 and Figure 7)**.

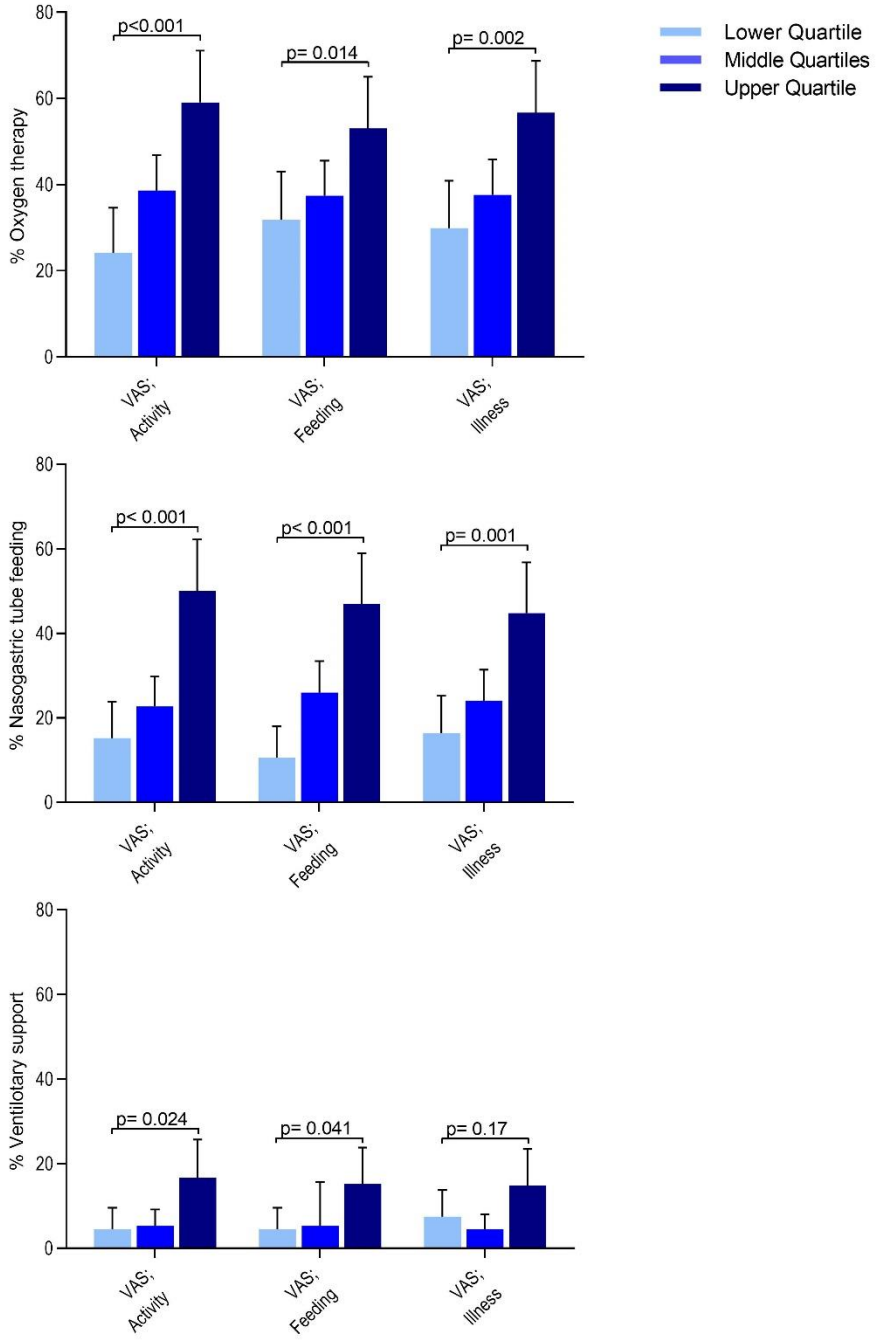
Figure 6 *Quartiles of parent VAS-scores and receiving supportive care during hospital stay*



Association between parental Visual Analogue Scale (VAS) score at inclusion and receiving supportive care during hospital stay in 267 infants with parental VAS score. Presented with 95% upper Confidence Interval. The level of significance is presented comparing upper and lower quartile.

From Acta Paediatrica, Gjengsto Hunderi JO, Lodrup Carlsen KC, Rolfsjord LB, Carlsen KH, Mowinckel P, Skjerven HO. Parental severity assessment predicts supportive care in infant bronchiolitis, Jan;108(1):131-13. Copyright © (2019).

Figure 7 *Quartiles of parent VAS-scores and receiving oxygen therapy, nasogastric tube feeding and ventilatory support during hospital stay.*



Association between parental Visual Analogue Scale (VAS) score at inclusion and receiving supportive care during hospital stay in 267 infants with parental VAS score. Presented with 95% upper Confidence Interval. The level of significance is presented comparing upper and lower quartile.

From Acta Paediatrica, Gjengsto Hunderi JO, Lodrup Carlsen KC, Rolfsjord LB, Carlsen KH, Mowinckel P, Skjerven HO. Parental severity assessment predicts supportive care in infant bronchiolitis, Jan;108(1):131-13. Online supplement. Copyright © (2019).

The parental VAS items were significantly associated with the use of supportive care during hospital stay, with Illness having the highest OR of 1.36 for receiving any supportive care **(Table 11).**

Respiratory rate and SpO_2 was also significantly associated with receiving supportive care during hospital stay.

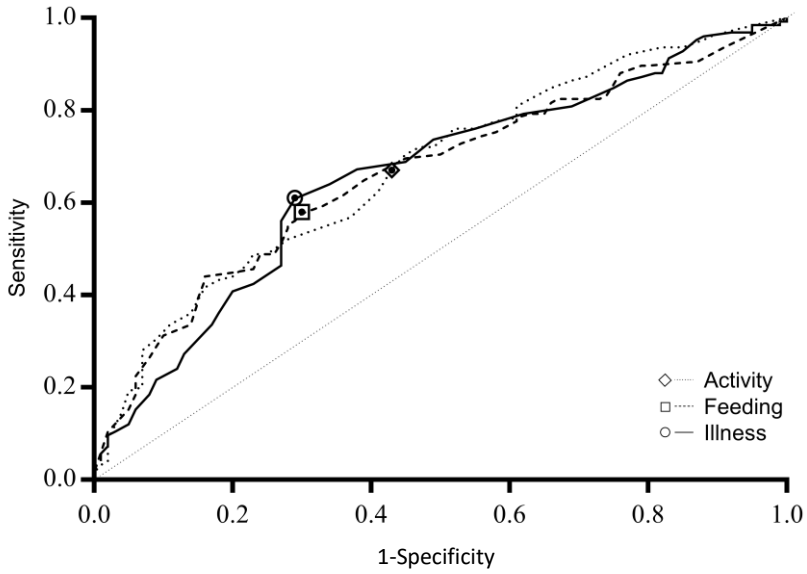
Table 11 *Odds ratios for receiving supportive care by severity assessments*

	Oxygen therapy OR (95% CI)	**Nasogastric tube feeding** OR (95% CI)	**Ventilatory support** OR (95% CI)	**Any supportive care** OR (95% CI)
Clinical Score	1.36 (1.12, 1.68)	1.51 (1.21, 1.86)	1.61 (1.15, 2.26)	1.28 (1.04, 1.56)
VAS Activity	1.22 (1.11, 1.35)	1.30 (1.16, 1.45)	1.23 (1.02, 1.49)	1.26 (1.15, 1.39)
VAS Feeding	1.14 (1.05, 1.23)	1.32 (1.19, 1.46)	1.28 (1.07, 1.52)	1.23 (1.13, 1.34)
VAS Illness	1.34 (1.17, 1.55)	1.40 (1.20,1.64)	1.35 (1.04, 1.75)	1.36 (1.18, 1.56)
Respiratory rate	1.01 (0.99, 1.04)	1.03 (1.01, 1.06)	1.0 (0.96, 1.05)	1.02 (1.0, 1.05)
SpO_2	0.77 (0.69, 0.85)	0.87 (0.80, 0.96)	0.73 (0.63, 0.84)	0.78 (0.70, 0.86)

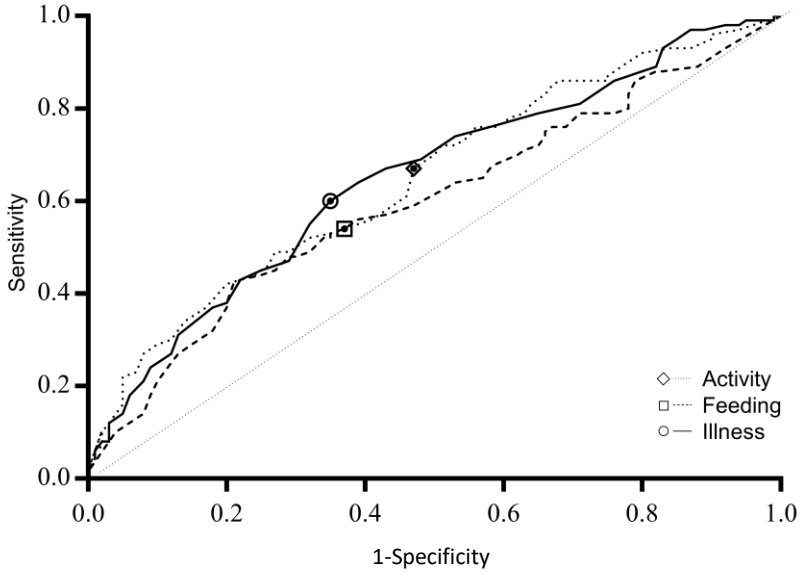
The table reports the Odds Ratio (OR) for infants 0-12 months of age admitted to hospital with acute bronchiolitis receiving supportive care based upon the clinical score and parental disease severity assessment by three visual analogue scale (VAS) items Activity, Feeding, Illness, respiratory rate and peripheral capillary oxygen saturation (SpO2) at the time of hospital admission. Bivariate OR are adjusted for age and sex. The clinical score and each VAS item scored from 0-10, 10 being most severe, while a lower SpO_2 denoted more severe disease.

Figure 8 *Receiver operating characteristic curves for parental VAS items at inclusion*

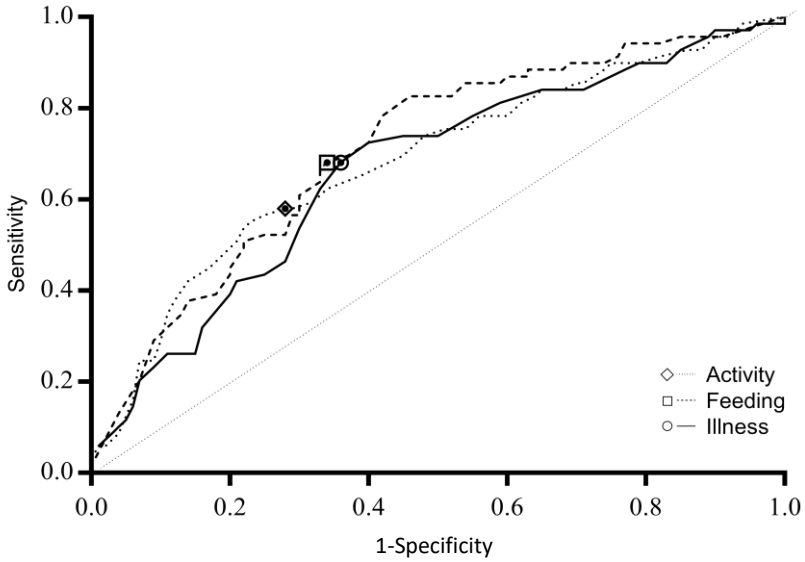
A) Any supportive care



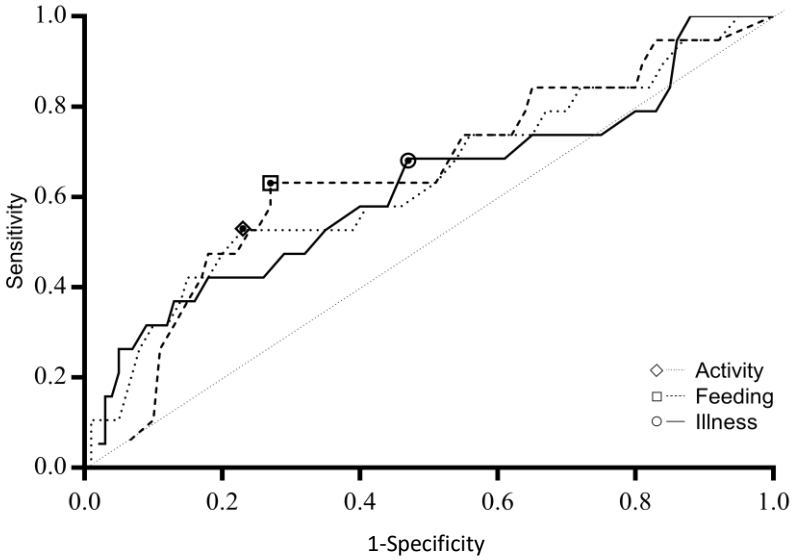
B) Oxygen therapy



C) Nasogastric tube feeding



D) Ventilatory support



The figures show receiver operating characteristic curves for the three visual analogue scale (VAS) items; Activity, Feeding and Illness at inclusion, predicting any supportive care (a), oxygen therapy (b), nasogastric tube feeding (c) and ventilatory support (d) in infants with bronchiolitis. Optimal cut off for each curve is marked. Area under the curve varied between 0.60 and 0.71 **(Table 12).**

From Acta Paediatrica, Gjengsto Hunderi JO, Lodrup Carlsen KC, Rolfsjord LB, Carlsen KH, Mowinckel P, Skjerven HO. Parental severity assessment predicts supportive care in infant bronchiolitis, Jan;108(1):131-13. Online supplement. Copyright © (2019).

The area under the receiver operating curve (AUC) defines the ability of each of the VAS items to predict the use of supportive care and ranged from 0.60 to 0.71 **(Table 12).**

Table 12 *Area under the curve for the parental VAS predicting supportive care*

	Oxygen therapy	**Nasogastric tube feeding**	**Ventilatory support**	**Any supportive care**
VAS Activity%	0.65	0.68	0.64	0.67
(95% CI)	(0.57, 0.71)	(0.60, 0.75)	(0.47, 0.76)	(0.60, 0.73)
VAS Feeding%	0.60	0.71	0.65	0.66
(95% CI)	(0.52, 0.66)	(0.63, 0.77)	(0.49, 0.76)	(0.59, 0.72)
VAS Illness %	0.65	0.67	0.62	0.66
(95% CI)	(0.57, 0.71)	(0.58,0.73)	(0.44, 0.75)	(0.59, 0.72)

Area under the receiver operating characteristic curve[a] for the three visual analogue scale (VAS) items; Activity, Feeding and Illness, predicting supportive care [a]Displayed in figure 8. P<0.05 for all

By the optimal cut-off values, illustrated in **Figure 8**, sensitivity, specificity and likelihood ratios were calculated **(Table 13)**. VAS item Illness was found to have the strongest association with supportive care with a sensitivity of 61% and specificity of 71%, followed by VAS feeding and VAS activity. The Clinical Score was found to have the lowest sensitivity, specificity and positive and negative likelihood ratios.

Table 13 *Sensitivity, specificity and likelihood ratios for predicting supportive care*

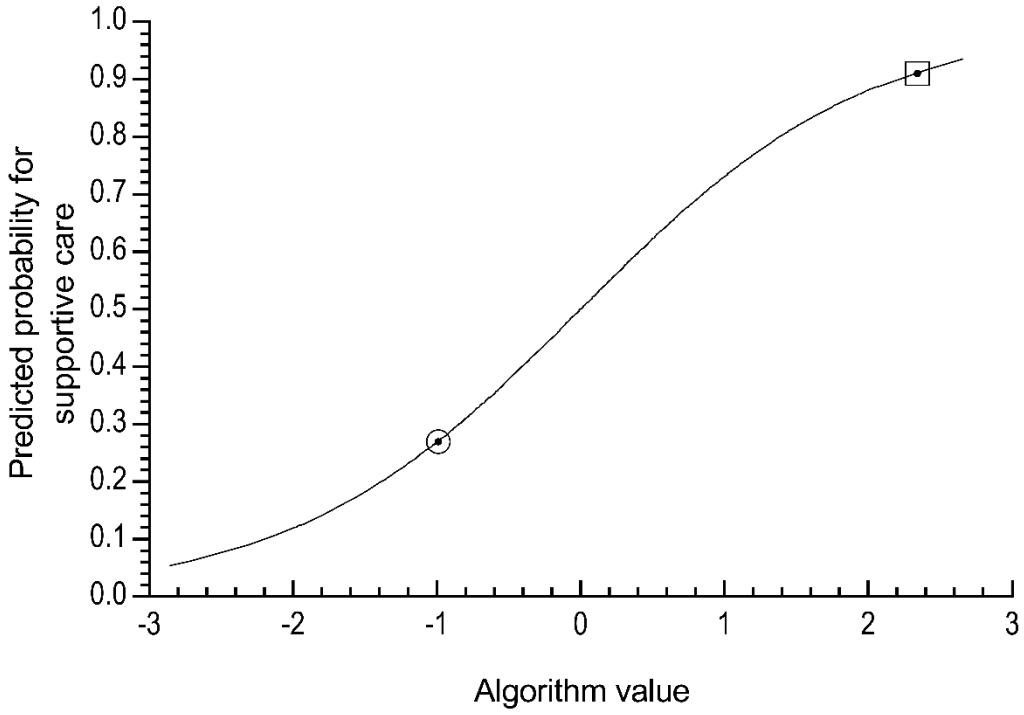
	Cut-off Value[a]	Sensitivity% (95% CI)	Specificity% (95% CI)	Pos. Likelihood Ratio	Neg Likelihood Ratio
Clinical Score					
Supportive care	5.0	*59 (50, 68)*	*45 (37, 54)*	1.07	0.91
Oxygen therapy	5.0	58 (48, 68)	44 (36, 52)	1.04	0.95
Nasogastric tube feeding	5.0	67 (54, 78)	47 (39, 54)	1.52	0.86
Ventilatory support	5.0	63 (38,84)	44 (37, 50)	1.33	0.98
VAS Activity					
Supportive care	5.0	67 (58, 75)	57 (48, 66)	1.67	0.63
Oxygen therapy	5.0	67 (67, 76)	53 (45, 61)	1.71	0.72
Nasogastric tube feeding	6.2	*58* (45, 70)	*72* (65, 79)	2.07	0.58
Ventilatory support	7.2	53 (29, 76)	77 (71, 83)	2.3	0.61
VAS Feeding					
Supportive care	5.8	58 (48, 66)	70 (62, 78)	1.93	0.6
Oxygen therapy	5.8	54 (44, 64)	63 (55, 71)	1.53	0.75
Nasogastric tube feeding	5.8	68 (56, 79)	66 (58, 73)	2.0	0.48
Ventilatory support	7.6	63 (38, 84)	73 (66, 78)	2.33	0.51
VAS Illness					
Supportive care	6.2	*61 (52, 69)*	*71 (61, 79)*	*2.1*	*0.55*
Oxygen therapy	6.2	60 (50, 70)	65 (57, 73)	1.71	0.62
Nasogastric tube feeding	6.2	68 (56, 79)	64 (57, 71)	1.89	0.5
Ventilatory support	6.0	68 (43, 87)	53 (46, 59)	1.45	0.6
Respiratory Rate					
Supportive care	51	64 (55, 72)	46 (37, 55)	1.19	0.78
Oxygen therapy	57	47 (40, 57)	60 (52, 68)	1.17	0.88
Nasogastric tube feeding	55	67 (54, 78)	52 (45, 60)	1.4	0.63
Ventilatory support	56	42 (20, 67)	53 (46, 59)	0.89	1.09
SpO_2					
Supportive care	96	*63 (54, 72)*	*63 (54, 71)*	*1.7*	*0.59*
Oxygen therapy	96	67 (57, 76)	61 (53, 69)	1.72	0.54
Nasogastric tube feeding	95	54 (41, 66)	72 (65, 79)	1.93	0.64
Ventilatory support	93	58 (34, 80)	88 (83, 92)	4.83	0.48

Sensitivity, specificity, positive and negative likelihood ratios for the clinical score, the three visual analogue scale (VAS) items; Activity, Feeding, Illness, respiratory rate and SpO_2 predicting supportive care.

[a] The cut-off values were stablished by receiver operating characteristic analyses (Online Figure 2)

The predicted probability curve for receiving supportive care included VAS- items Feeding and Illness, SpO_2, age and gender. The discriminant ability of the prediction algorithm to predict use of supportive care during hospital stay given by AUC was 0.76 (95% CI: 0.69- 0.81), $p<0.001$. The highest combined sensitivity of 81% (73%, 87%) and specificity of 61% (52%, 69%) was found with a cut-off value of ≥ -0.18. This gives a positive and negative likelihood ratio of 2.1 and 0.31 and positive and negative predictive values of 68% (60%, 75%) and 76% (66%, 84%), respectively.

Figure 9 *Predicted probability curve for receiving supportive care*



The predicted probability curve for receiving supportive care is based on the parental visual analogue scale (VAS) items Feeding and Illness, SpO2, age and gender. Once the algorithm value is calculated using the algorithm; 17.9 + 0.16×VAS Feeding + 0.19×VAS Illness – 0.21 × SpO_2 + Gender (male=1, female =2) × 0.62 - Age (days)×0.004, the individual probability of receiving supportive care is estimated from the probability curve.
Example 1 (circle): Using median VAS of the lower quartile, male gender and mean age.
VAS Feeding: 0.6, VAS Illness: 3.4, SpO_2 (%): 94, Male: value 1, Age (days): 128, gives an algorithm value of -0.99 with a predicted probability of receiving supportive care of 27%.
Example 2 (square): Using median VAS of the upper quartile, female gender and young age.
VAS Feeding: 9.4, VAS Illness: 8.2, SpO_2 (%): 94, Female: value 2, Age (days): 30, gives an algorithm value of 2.34 with a predicted probability of receiving supportive care of 91%.

From Acta Paediatrica, Gjengsto Hunderi JO, Lodrup Carlsen KC, Rolfsjord LB, Carlsen KH, Mowinckel P, Skjerven HO. Parental severity assessment predicts supportive care in infant bronchiolitis, Jan;108(1):131-13. Copyright © (2019).

4.3 Severity of acute bronchiolitis and early asthma development. (Papers #2, 4)

At the 2-year follow-up investigation, 143 of the 294 children (49%) had recurrent wheeze and 56 (19%) were diagnosed with asthma by a physician.

There were no significant differences in clinical score, parental VAS score at hospitalization, LOS or the use of any supportive care in infancy among children who at two years had recurrent wheeze compared to no recurrent wheeze, and no significant associations were observed in bivariate logistic analyses **(Table 14).**

Table 14 *Severity of acute bronchiolitis for children with recurrent wheeze at two years of age compared to those with no recurrent wheeze and Odds Ratios (OR) for having recurrent wheeze at two-years of age based on bronchiolitis severity. All analyses adjusted for age and sex at inclusion.*

	Recurrent wheeze n= 143 (48.6)	**No recurrent wheeze n= 151 (51.4)**	*p*	**Recurrent Wheeze OR (95% CI)**	*p*
Clinical score (SD)	4.8 (1.0)	5.0 (1.1)	*0.55*	0.88 (0.70, 1.10)	*0.27*
VAS Activity (SD)	5.0 (2.7)	5.5 (2.9)	*0.97*	1.01 (0.91, 1.11)	*0.92*
VAS Eating (SD)	5.7 (3.2)	4.8 (3.5)	*0.06*	1.09 (1.0, 1.19)	*0.06*
VAS Illness (SD)	6.1 (1.9)	5.8 (2.0)	*0.25*	1.09 (0.95, 1.26)	*0.23*
Length of hospital stay, hours (SD)	85 (72)	76 (59)	*0.16*	1.0	*0.12*
Oxygen therapy, n (%)	61/135 (45.2)	63/145 (43.3)	*0.77*	1.22 (0.75, 1.99)	*0.43*
Nasogastric tube feeding, n (%)	36/142 (25.4)	50/149 (33.6)	*0.13*	0.72 (0.43, 1.21)	*0.22*
Ventilatory support, n (%)	10/143 (7.0)	9/151 (6.0)	*0.72*	1.18 (0.46, 3.01)	*0.73*
Receiving supportive care, n (%)	74/143 (51.7)	79/ 151 (52.3)	*0.92*	1.08 (0.68, 1.73)	*0.74*

Applying multivariate logistic analyses with Hosmer stepdown procedure, including variables from table 14, no significant associations were detected.

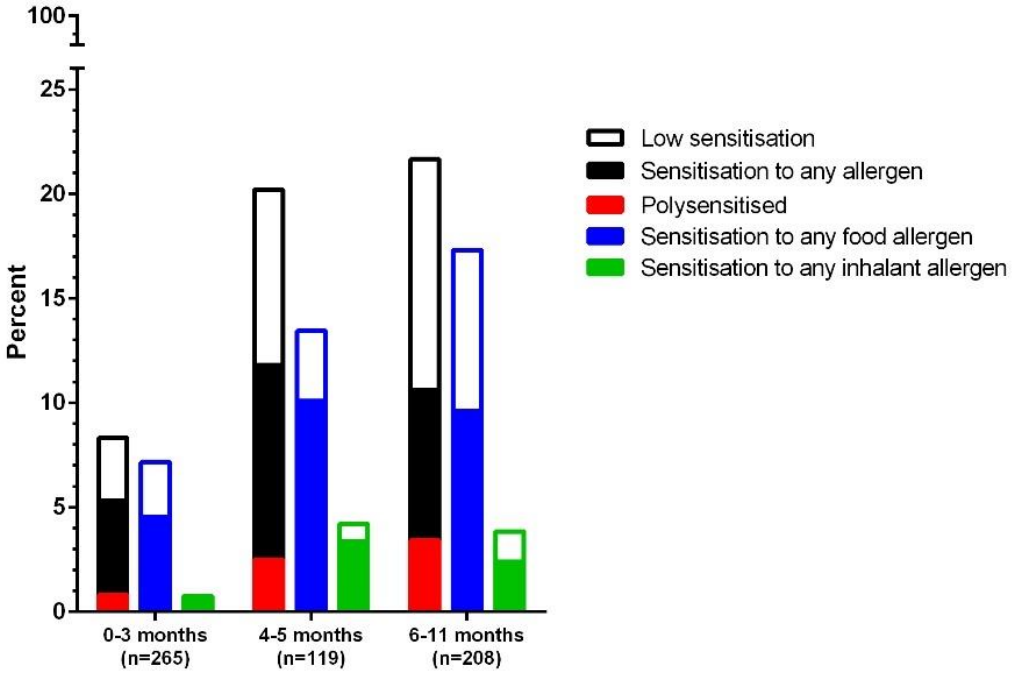
4.4 To determine prevalence of early allergic sensitization and the role of allergic sensitisation, type or load of viruses or salivary cortisol during acute bronchiolitis in infancy for asthma development. (Papers # 2, 3, 4)

4.4.1 Allergic sensitisation

Serum IgE- analysis at enrolment was available for 592 infants at a mean age of 5.1 months, with 368 from the bronchiolitis group (mean age 4.2 months) and 224 i from the control group (mean age of 6.6 months).

Overall, 86 infants (14.5%) had a Phadiotop infant of ≥0.15 kU/l while 8.5% were sensitised (s-IgE ≥0.35 kU/l) to at least one allergen, and low allergic sensitisation (s-IgE 0.1- 0.34 kU/l) was observed in an additional 6.9%. We observed no significant difference in allergic sensitisation between the bronchiolitis group (8.4%) and the control group (8.5%). Allergic polysensitisation, sensitised (s-IgE ≥0.35 kU/l) to more than on allergen, was observed in 2% of the infants.

Figure 10 Allergic sensitisation, cross sectional analyses in an infant population



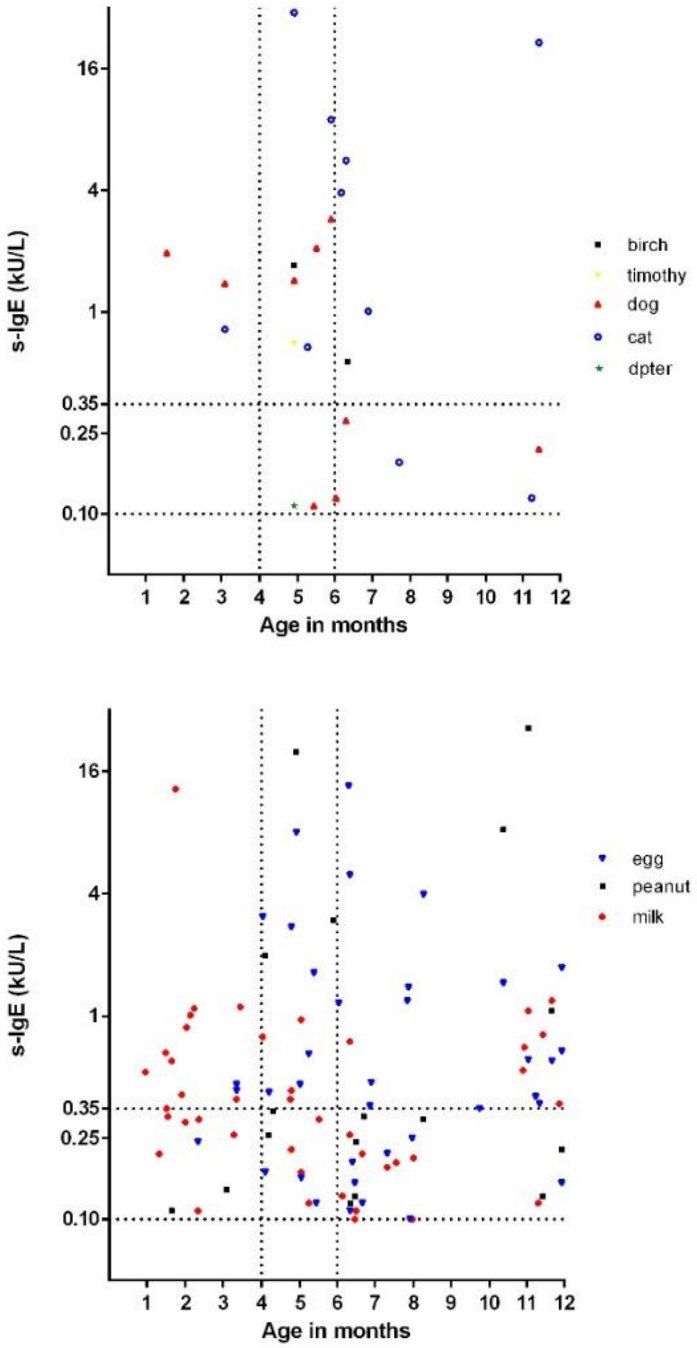
Low allergic sensitisation is defined as s-IgE of 0.10-0.34 kUA/l and is displayed with borders only. Allergic sensitisation is defined as s-IgE ≥0.35 kU/L. Food allergens include egg, milk and peanut. Inhalant allergens include birch, timothy, cat, dog, d. pteronyssinus, cladiosporum and guinea pig.

From Pediatr Allergy Immunol, Skjerven HO, Hunderi JOG, Carlsen KH, Rolfsjord LB, Nordhagen L, Berents TL, Bains KES, Buchmann M, Carlsen KCL. Allergic sensitisation in infants younger than one year of age, Feb;31(2):203-206. Copyright © (2020).

Infants younger than four months of age were significantly less often sensitised compared to the two oldest age groups (p=0.01) **(Figure 10)**. Sensitisation to food allergens was most common (7.4%), with sensitisation to egg being most common, followed by cow's milk and peanut. In infants younger than 4 months old, sensitisation to cow's milk was most frequent. Only 1.9 children were sensitised to inhalant allergens, with cat being the most frequent, followed by dog, birch, timothy and house dust mite **(Figure 11).**

There were no significant differences in allergic sensitisation in infants with, compared to without, parental allergic rhinitis (9.2% vs 9.1%) or parental asthma (8.4% vs 8.7% respectively), nor between boys (8.1%) and girls (7.4%).

Figure 11 *Sensitisation levels are given for each individual for inhalant allergens (top) and food allergens (below), by age in months.*



Each point represents a single sensitisation value. Each individual may be represented several times. Levels below 0.10 kUA/l are not displayed. Dotted x-axis lines represent low sensitisation (0.10 kU/l) and sensitisation (≥0.35 kU/L).

From Pediatr Allergy Immunol, Skjerven HO, Hunderi JOG, Carlsen KH, Rolfsjord LB, Nordhagen L, Berents TL, Bains KES, Buchmann M, Carlsen KCL. Allergic sensitisation in infants younger than one year of age, Feb;31(2):203-206. Copyright © (2020).

4.4.2 Asthma development

There were no significant differences in virus detection, allergic sensitisation or cortisol levels between children with recurrent wheeze and no recurrent wheeze at two years of age, as shown in **Table 15** together with baseline and clinical characteristics.

Children at two years of age with recurrent wheeze had significantly more often a doctor's diagnosis of asthma (35.7%) as well as higher extent of use of asthma medication (80.6% versus 19.7%) than those with no recurrent wheeze (3.3%) **(Table 15).**

Table 15 *Baseline and clinical characteristics of the 294 children are shown for the 143 children with and 151 with no recurrent at two years of age*

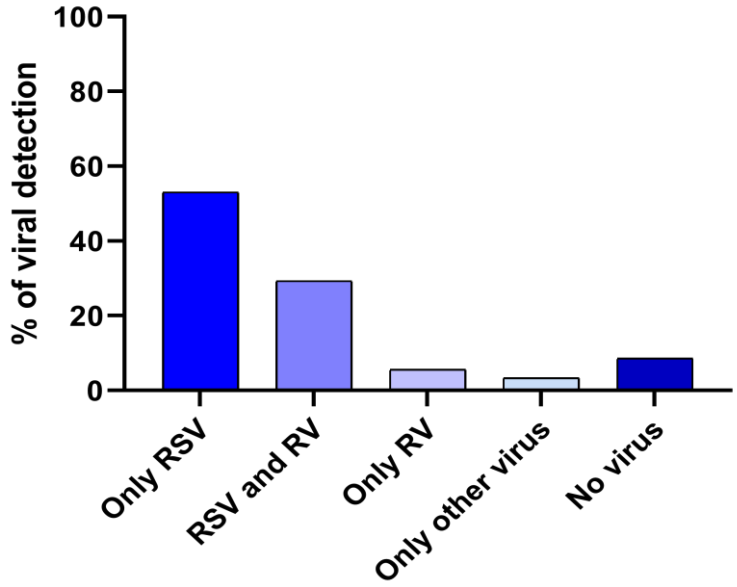
		Recurrent wheeze n= 143 (48.6)	No recurrent wheeze n= 151 (51.4)	p
At birth	*Male sex n (%)*	96/143 (67.1)	85/ 151 (56.3)	0.06
	Gestational age (GA) weeks (SD)	38.6 (2.4)	38.5 (4.0)	0.95
	Born at GA<37 weeks, n (%)	14/110 (12.7)	15/116 (12.9)	0.96
	Caesarion section delivery, n (%)	36/130 (27.7)	25/138 (18.1)	0.06
	Birth weight, grams (SD)	*3347 (632)*	*3497 (642)*	0.05
Study enrolment/ hospital stay				
	Age, days (range)	134 (14, 348)	115 (7, 363)	0.05
	Weight, grams (SD)	6650 (1886)	6245 (1794)	0.06
	Eczema n (%)	15/131 (11.5)	14/ 140 (10.0)	0.70
	One previous episode of wheeze (%)	34/127 (26.8)	35/133 (26.3)	0.93
	Length of hospital stay, hours (SD)	85 (72)	76 (59)	0.24
	Receiving supportive care, n (%)	74/143 (51.7)	79/ 151 (52.3)	0.92
Virus detected during acute bronchiolitis				
	Respiratory syncytial virus (RSV), n (%)	107/129 (82.9)	112/ 137 (81.8)	0.80
	Human rhinovirus (HRV), n (%)	45/129 (34.9)	48/137 (35.0)	0.98
	HRV A or B, n (%)	12/129 (9.3)	16/137 (11.7)	0.53
	HRV C, n (%)	33/129 (25.6)	32/137 (23.4)	0.67
	RSV, high genomic load, n (%)	68/129 (52.7)	77/ 137 (56.2)	0.57
	HRV, high genomic load, n (%)	8/129 (6.2)	8/137 (5.8)	0.90
	More than 1 virus, n (%)	80/129 (62.0)	90/ 137 (65.7)	0.53
Allergic sensitisation, IgE ≥ 0.35 kU/l				
	Any sensitisation, n (%)	9/130 (6.9)	13/141 (9.2)	0.49
	Any food sensitisation, n (%)	8/130 (6.2)	12/141 (8.5)	0.46
	Any inhalant sensitisation, n (%)	3/130 (2.3)	2/141 (1.4)	0.59
	Egg sensitisation, n (%)	5/130 (3.8)	3/141 (2.1)	0.40
	Cow's milk sensitisation, n (%)	4/130 (3.1)	9/141 (6.4)	0.20
	Peanut sensitisation, n (%)	1/141 (0.7)	2/130 (1.5)	0.52
	Polysensitisation, n (%)	4/130 (3.1)	3/141 (2.1)	0.62
	Monosensitisation, n (%)	4/130 (3.1)	9/141 (6.4)	0.20
Salivary cortisol				
	geometric mean, mmol/l (95% CI)	37.0 (30.2, 45.4)	33.7 (28.5, 39.8)	
At the two-year follow-up				
	Age, days (range)	747 (291, 1055)	725 (368, 979)	0.07
	Asthma diagnosed by physician, n (%)	51/ 143 (35.7)	5/151 (3.3)	**<0.001**
	Asthma medication used, n (%)	108/134 (80.6)	28/142 (19.7)	**<0.001**
Parental education				
	Maternal Education[a] (SD)	3.93 (0.94)	4.05 (1.03)	0.31
	Paternal Education[a] (SD)	3.76 (0.97)	3.92 (1.02)	0.21
Parental allergic diseases				
	Any[b] n (%)	70/128 (54.7)	60/136 (44.1)	0.09
	Maternal Asthma, n (%)	22/112 (19.6)	14/123 (11.4)	0.08
	Paternal Asthma, n (%)	16/112 (14.3)	15/123 (12.2)	0.64
	Maternal Rhinoconjuncivitis, n (%)	21/124 (16.9)	21/ 134 (15.7)	0.78
	Paternal Rhinoconjuncivitis, n (%)	31/124 (25.0)	25/134 (18.7)	0.22
	Maternal eczema, n (%)	20/128 (15.6)	11/135 (8.1)	0.06
	Paternal eczema, n (%)	15/128 (11.7)	10/135 (7.4)	0.23
Environment				
	Smoking at home n (%)	19/124 (15.3)	19/129 (14.7)	0.90

[a] Education was categorised from 1 (no school completed) to 5 (higher education, more than three years)

[b] Defined by reported asthma, eczema and rhinoconjunctivitis

Respiratory syncytial virus was detected in 82% and HRV in 35% of the infants hospitalised with acute bronchiolitis **(Figure 12)**, while high genomic load was observed in 54% for RSV and 6% for HRV.

Figure 12 *The distribution of the viruses analysed in 266 infants hospitalized with bronchiolitis disease*



(RSV 53%, HRV and RSV 29.3%, HRV 5.6%, other viruses 3.4%, no virus detected 8.6%)

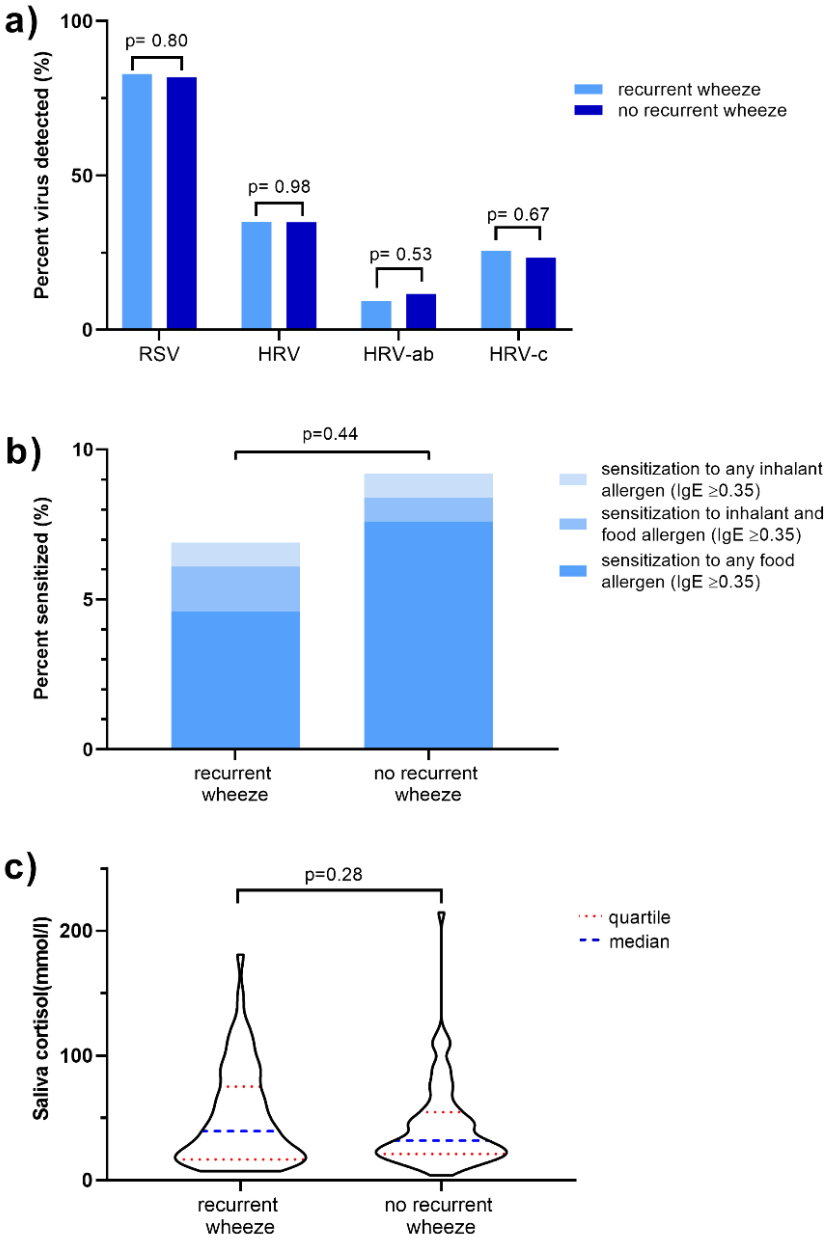
From European Respiratory Journal open research, Hunderi JOG, Rolfsjord LBD, Lødrup Carlsen KC, Holst R, Bakkeheim E, Berents TL, Carlsen KH, Skjerven HO. Virus, allergic sensitisation and cortisol in infant bronchiolitis and risk of early asthma. ERJ Open Res 2020; 6: 00268-2019. *Copyright © (2020).*

There were no significant differences in infective viral agents during acute bronchiolitis when comparing children with and without recurrent wheeze at two years of age **(Table 15 and Figure 13)**.

There were no significant differences in allergic sensitisation to any allergen nor in displaying low allergic sensitisation in infancy among children with and without recurrent wheeze at two years of age **(Table 15, Table 16, Figure 13)**.

The geometric mean salivary cortisol level sampled on the first morning after hospitalisation was similar among infants with (37.0 mmol/l) and without (33.7 mmol/l) recurrent wheeze at two years of age **(Table 15 and Figure 13)**.

Figure 13 *Distribution of virus, sensitisation and cortisol comparing children with and without recurrent wheeze at two years of age.*



a) the distribution of Respiratory Syncytial Virus (RSV), Human Rhino Virus (HRV), HRV species A and B (HRV- ab) and HRV species C (HRV- c) b) the distribution of allergic sensitisation to inhalant and food allergens and c) the distribution of morning saliva cortisol sampled first morning after hospitalisation, in infants hospitalised with acute bronchiolitis (study enrolment) 0- 12 months of age (mean age 4.2 months), comparing children with recurrent wheeze and no recurrent wheeze at two- years follow-up.

From European Respiratory Journal open research, Hunderi JOG, Rolfsjord LBD, Lødrup Carlsen KC, Holst R, Bakkeheim E, Berents TL, Carlsen KH, Skjerven HO. Virus, allergic sensitisation and cortisol in infant bronchiolitis and risk of early asthma. ERJ Open Res 2020; 6: 00268-2019. *Copyright © (2020).*

Table 16 *Distribution of low allergic sensitisation among children with and without recurrent wheeze at two years of age*

	Recurrent wheeze n= 143 (48.6)	No recurrent wheeze n= 151 (51.4)	p
Low Allergic sensitisation			
Any sensitisation, n (%)	18/130 (13.8)	17/141 (12.1)	0.66
Any food sensitisation, n (%)	18/130 (13.8)	17/141 (12.1)	0.66
Any inhalant sensitisation, n (%)	15/129 (11.6)	14/138 (10.1)	0.70
Egg sensitisation, n (%)	18/130 (13.8)	17/141 (12.1)	0.66
Cow's milk sensitisation, n (%)	15/130 (11.5)	16/141 (11.3)	0.96
Peanut sensitisation, n (%)	15/130 11.5)	15/141 (10.6)	0.81

Low sensitisation is defined by IgE 0.10- 0.34 kU/l before one year of age. Shown for the 294 infants hospitalised with bronchiolitis and attending two- year follow-up.

We found no significant association between specific virus, high viral load of RSV or HRV, allergic sensitisation or morning salivary cortisol level during acute infant bronchiolitis and recurrent wheeze at two years of age **(Table 17)**, adjusting for age, sex, parental atopy and time since enrolment. There were no significant interactions between specific viruses, viral load, allergic sensitisation and salivary cortisol levels.

Table 17 Odds Ratios (OR) and Incidence rate ratio (IRR) for infants 0-12 months of age with acute bronchiolitis of having recurrent wheeze at two-years of age, based on viral detection, early sensitisation and morning salivary cortisol level, Adjusted for gender, age at inclusion and parental atopy.

	Recurrent wheeze OR (95% CI)	p	**Recurrent wheeze** IRR (95% CI)	p
Viral detection during acute bronchiolitis				
RSV	0.91 (0.45, 1.83)	0.78	0.95 (0.70, 1.28)	0.86
HRV	1.03 (0.59, 1.78)	0.93	0.72 (0.57, 0.91)	0.16
HRV A or B	0.74 (0.31, 1.8)	0.51	0.58 (0.40, 0.83)	0.14
HRV C	1.19 (0.65, 2.19)	0.58	0.84 (0.65, 1.09)	0.51
RSV, high genomic load	0.84 (0.50,1.42)	0.52	0.92 (0.73, 1.15)	0.70
HRV, high genomic load	0.93 (0.30, 2.84)	0.90	0.61 (0.38, 0.99)	0.31
Multiple viruses	0.83 (0.47, 1.44)	0.50	0.91 (0.72, 1.16)	0.69
Allergic sensitisation, IgE ≥ 0.35				
Any sensitisation	0.72 (0.28, 1.89)	0.51	1.46 (0.97, 2.19)	0.36
Any food sensitisation	0.71 (0.25, 1.96)	0.50	1.53 (1.0, 2.36)	0.32
Any inhalant sensitisation	1.08 (0.19, 20.89)	0.56	1.18 (0.48, 2.90)	0.85
Egg sensitisation	1.33 (0.27, 6.45)	0.72	3.58 (1.82, 3.58)	0.06
Cow's milk sensitisation	0.41 (0.10, 1.64)	0.21	0.49 (0.29, 0.95)	0.17
Peanut sensitisation	0	0.99	1.45 (0.25, 8.33)	0.83
Polysensitisation	1.61 (0.29, 32.79)	0.35	1.44 (0.60, 3.49)	0.68
Salivary morning cortisol	1.00 (0.99, 1.01)	0.40	1.01 (1.0, 1.0)	0.56

We found no significant effects on the incidence rate ratios for recurrent wheeze by specific viruses, high viral load of RSV or HRV, allergic sensitisation or morning salivary cortisol level, applying zero truncated negative binomial regression analysis adjusting for age, sex, parental atopy and time since enrolment **(Table 17).**

5 Discussion

5.1 Risk factors for receiving supportive care for acute moderate to severe infant bronchiolitis.

In our study, Caesarion section delivery, SpO_2 < 92%, increased heart rate and lower age at hospitalisation were found to increase the risk for receiving supportive care during hospital stay, explaining only about 12% of factors related to receiving supportive care.

The use of supportive care in 50% of the infants hospitalised with acute bronchiolitis (41% oxygen therapy, 29% nasogastric tube feeding and 7% CPAP) in the Bronchiolitis ALL SE Norway study is in line with other studies (56, 106-108). Wainwright and co-workers reported that 44.8% of infants less than 12 months hospitalised with bronchiolitis received oxygen therapy (56) and in 492 infants younger than two months hospitalised with acute bronchiolitis in Australia, 30% received nasogastric tube feeding (106). The use of CPAP during acute bronchiolitis was reported for 7.9% of infants in another Norwegian study (107) while in the United States, 7% of children age <2 years received CPAP and/or were intubated (108) .

Caesarean delivery is associated with early respiratory distress, but may also predispose for respiratory complications later in childhood (109).

Peripheral capillary oxygen saturation with SpO_2 <98% at hospital admission in our study was significantly associated with receiving supportive care. An SpO_2 <92%, observed in 29 (7.7%) of infants at enrolment, gave the highest OR of 5.3 for receiving supportive care, with 80% receiving supplementary oxygen. Only 18 infants (4.6%) presented with an SpO_2 <90% at hospital admission, of whom 83% received oxygen therapy during their hospital stay. The best cut-off value for SpO_2 was 96% with a sensitivity and specificity of 63% for receiving any supportive care. Even if a SpO_2 cut-off of 92% gave a higher OR, few infants had a value lower than 94%, and 70% of infants receiving any supportive care had an SpO_2 on admission of >96%. Using SpO_2 only to guide admission would

potentially lead to the failure to discover 37% of the infants who actually needed supportive care. Corneli and coworkers identified SpO_2 <94%, respiratory rate >60/min and Respiratory Distress Assessment Instrument (RDAI) (40) score 11< to be the best predictors of hospitalisation, with a sensitivity of 56% and a specificity of 74%. However, a sensitivity of 56% would indicate that 44% of infants would be falsely assessed to have a good prognosis, while a specificity of 74% would mean that 26% of infants would be erroneously projected a worse prognosis.

Measures of SpO_2 have been used since the 1980s (110), are recognised as the fifth vital sign (111, 112) and may have contributed to the increase in hospitalisation rates for bronchiolitis seen in the last decades (113, 114) with mortality rates being relatively constant (115). The American Academy of Pediatrics (AAP) guideline postulates that "pulse oximetry has been erroneously used in bronchiolitis as a proxy for respiratory distress" (65). A definition of what constitutes normal SpO_2 values has not yet been established, and there is no consensus on what level to use in the clinical decision making in infants with acute bronchiolitis; when to admit, treat or discharge from hospital (31, 65, 116). An Australian literature review revealed significant knowledge deficits about pulse oximetry amongst nurses and physicians (117).

The National Institute for Health and Care Excellence (NICE), on the subject of how to diagnose and manage infants and children with bronchiolitis, recommend that all infants with acute bronchiolitis who have SpO_2 ≤92% should be admitted to hospital (31). Likewise, the British Thoracic Society guideline recommends that symptomatic infants and children with community-acquired pneumonia and SPO_2 ≤92% should be hospitalised (116). The AAP guidelines do not recommend oxygen therapy before SpO_2 levels persistently fall below 90% in previously healthy infants with acute bronchiolitis.

In our study, all 404 infants with acute bronchiolitis were admitted to hospital, thus we are unable to determine the role of SpO_2 threshold for deciding whether or not to admit the infant to hospital. However, among the 162 infants treated with oxygen therapy, 70% had SpO_2 >94% at hospital admission, pointing to a high sensitivity, but lower specificity of a SpO_2<92% on presenting to the

hospital. Although SpO_2 at hospital admission was significantly associated with the use of supportive care, especially oxygen therapy, levels of SpO_2 >90- 92% should be used with caution in clinical decision making.

Increased heart rate at hospital admission was identified as a predictor of receiving supportive care during hospital stay. This is in line with a study by Walsh and co-workers (32) identifying pre-treatment tachycardia, defined by heart rate >149, to be more frequent among children, age <2 years, hospitalised 4< days compared to those hospitalised <4 days and non-hospitalised children. The final prediction model by which to ascertain need for admission and length of hospital stay also included age, dehydration, and increased work of breathing predicting admission with sensitivity and specificity of 91% and 83% respectively. Among 29 potential clinical predictors of hospital admission, Marlais (45) et al. identified heart rate, respiratory rate, age, oxygen saturation and duration of symptoms to be the best predictors (45). Heart rate gave an OR (95% CI) of 1.05 (1.03, 1.06), p<0.001 with a ROC (95% CI) of 0.72 (0.67, 0.77).

In our study, younger age at hospitalisation was significantly associated with receiving supportive care, in line with previous reports that younger age is a recognized risk factor for developing a more severe bronchiolitis also in previously healthy infants (8, 31, 109, 118). This may be related to more immature lungs, with higher airway resistance in the youngest infants (109). Young infants with bronchiolitis is of higher risk of developing apnoea which is associated with need of respiratory support (8), and the NICE guidance recommends infant <3 months to be referred to hospital for further evaluation (31).

The variables from the multivariate analyses explaining only around 12% of the factors related to receiving supportive care during hospital stay with acute bronchiolitis, illustrates the limited utility of some clinical scores. It also points out that there are several factors being beyond our control affecting the course of the bronchiolitis.

In the univariate analyses adjusted for age and gender, premature birth, high respiratory rate (≥60 per minute), RSV high genomic load was significantly associated with receiving supportive care but did not reach statistical significance in the multivariate analysis.

It was previously reported that infants born prematurely were at risk of more severe bronchiolitis (8, 12), with increased need of hospitalisation with decreasing GA (119).

Infants with a high respiratory rate (≥60 per minute) had a greater risk of receiving supportive care than infants with lower respiratory rates. Respiratory rate is an important vital sign widely used to assess the cardiorespiratory status in children (112) and included in several clinical scores assessing disease severity (36, 37, 39, 42). Several studies have identified increased respiratory rate as a significant predictor of hospital admission (36, 44, 45). Among the 21 components in the Pediatric Risk of Admission (PRISA) score, elevated respiratory rate is one of the most important components calculating the probability of hospital admission (36). Corneli and co-workers found a respiratory rate greater than 60 per minute to be a significant predictor of hospitalisation with an OR (95% CI) of 2.6 (1.7, 4.1) (44).

Infants with RSV high genomic load were at greater risk of receiving supportive care, identified in 78% of the infants receiving ventilatory support. In a previous paper by Skjerven et al., high RSV genomic load was associated with longer LOS (16). Both supportive care and LOS may be a proxy for disease severity.

High RSV load is previously shown to be associated with respiratory failure (120) and bronchiolitis severity (28). DeVincenzo and colleagues have suggested that both airway size, immune response and lung structure interact with RSV load affecting disease severity (28). Based on our findings and those of others (16, 28), early recognition of viral load may therefore be useful in clinical decision making. However, determining high viral load, as was performed by cluster analysis in the present study, is time consuming and may not be available in all hospital laboratories. We found no

significant associations between type of virus or multiple viruses and receiving supportive care during hospitalisation.

To the best of our knowledge, the Bronchiolitis ALL study was the first to show that receiving inhalation therapy on demand appeared beneficial regarding disease severity and LOS compared to fixed schedules, while we found no significant associations related to supportive care and LOS by inhaled racemic adrenaline versus inhaled saline. The decision to give inhalations was made based on clinical judgment, primarily by nurses, but also by the attending physicians, probably resulting in fewer interruptions during sleep, in case of no signs of severe respiratory distress. Infants receiving inhalation therapy on demand received significantly fewer inhalations and less oxygen therapy and respiratory support by CPAP. Our findings support the recommendation that every handling procedure should be scrutinized and kept as simple and brief as possible. We are unaware of other studies addressing the efficiency of different inhalation strategies.

5.2 To determine if clinical scores or parental assessment of acute infant bronchiolitis at the time of hospitalisation predicts the short-term prognosis

In the Bronchiolitis ALL study, severity assessment at hospital admission by both a clinical score and parental VAS items were associated with short-term prognosis, measured by LOS and receiving supportive care.

Management with supportive care is the main reason for hospital admission of infants with acute bronchiolitis (12). The decision as to whether to admit an infant to hospital when they do not need supportive care may be challenging, based on the knowledge that some infants may deteriorate in line with the highly variable clinical course of acute bronchiolitis (12). The fact that around 50% of infants did not receiving supportive care, in line with other studies (56, 106-108) points to the need for precise tools to predict the need for supportive care, in order to reduce unnecessary hospitalisations (9, 10). However, this requires tools with both reliable positive and negative predictive values. General severity scores (36-38) and more specific bronchiolitis severity scores (32, 39-42) have been developed to guide hospital management. Severity might be defined as "an inherent characteristic of an illness, which reflects the natural history , that is, the prognosis in the absence of interventions" (121).

Although the clinical score in the Bronchiolitis ALL study, including respiratory rate, chest recessions, breathing sounds, skin colour and general condition at hospital admission predicted both LOS and the use of supportive care, the clinical value is limited due to the rather low positive likelihood ratio of 1.07 and negative likelihood ratio of 0.91, with respective sensitivity and specificity of 59% and 45% predicting supportive care.

The RDAI (40) evaluating the degree of wheeze and retractions, is thought to best reflect the underlying pathophysiology and has been found to have good interobserver agreement (40). However, with an AUC of 0.51 predicting hospital admission only (52), this tool is not helpful in predicting the need for hospital management. As previously described in chapter 5.1, Walsh and

coworkers combined subjective and objective variables in a model predicting need for hospital admission and LOS, with sensitivity of 91% and specificity of 83% (32).

A limitation of several scoring tools is that the outcomes were admission to hospital (45, 121), rather than a more accurate definition of disease severity as the basis for need of supportive treatment and LOS. Scores based on physical findings and respiratory examinations have been criticized for the lack of reproducibility, interobserver variability and laborious work (43). Evaluation of wheeze, thoracic retractions, nasal flaring and mental status are found to have poor interobserver reliability assessing a child with dyspnea (43, 46), postulating that the use of objective parameters might reduce this interrater variability.

Rating scales or clinical scores may be useful in the clinical practice and research, provided that they offer sufficient reliability (reproducible), validity and utility (46, 122). Clinicians and researchers should be able to draw similar conclusion from the measurements, and the measurements should be feasible (122) and suitable for use in children (46). None of the scores used to assess children with respiratory difficulties during asthma exacerbations (123-125) acute wheeze (58, 126) or acute bronchiolitis (39, 40, 42, 57) have been found to fulfill these demands (46).

Parental assessment of disease severity in children with acute bronchiolitis at hospital admission has not previously been studied, to our knowledge. All parental VAS items assessing Activity, Feeding and Illness at hospital admission for acute infant bronchiolitis were significantly associated with the use of supportive care. The parental VAS item Illness was superior to the clinical score in predicting use of supportive care overall, with sensitivity of 61% and specificity of 71% compared to the corresponding values for the clinical score of 59% and 45%, respectively.

Although we are unaware of parental assessments of their infant's clinical condition being evaluated for predicting short-term prognosis of acute bronchiolitis, our findings are supported by the report by Van den Bruel and co-workers identifying parental concern as an important diagnostic feature in

severity perception of infectious disease in children (47). Parents are thought to take other aspects into consideration than medical professionals (127). The decision to admit an infant with bronchiolitis is based on clinical evaluations (32), and the infant undergoes several examinations by the physicians on duty and nurses before the decision is made.

The child's ability to feed normally is identified as an important factor in the decision to hospitalise (52) and as a clinical marker of safe hospital discharge (128). History of poor feeding is also associated with increased LOS (32). None of the clinical scores previously described include the child's feeding ability or "eager to eat" status. Based upon our results, the parental assessments of their infant feeding may contribute positively to clinical decision making.

The parental interpretations of symptoms depend on the child's normal behaviour and parental anxiety about specific symptoms (129). In a study by MacFaul et al. comparing parents and physicians perception of need for hospital admission, they found parents rate illness severity higher than clinicians and found hospitalisation necessary even in cases of less severe disease (130). In contrast, parents were found to provide comparable assessments of acute asthma severity to respiratory therapists (131).

The sensitivity and specificity together with the AUC of 0.6- 0.71 indicate modest prediction. The predicted probability curve **(Figure 9)** including VAS feeding, VAS Illness, SpO_2 at hospital admission together with sex and age had an AUC of 0.76, indicating moderate to good ability to predict the use of supportive care.

Our study indicates that an overall parental assessment of activity level, feeding and general condition by VAS may strengthen the clinical decision making. In the complex nature of bronchiolitis, no scoring tool is likely to perform well (44), and conclusions regarding the decision to admit or discharge should not be based solely on a score itself. Both objective and subjective variables should be included in the final clinical decision making, together with the evaluation of parents and other healthcare providers.

5.3 Severity of acute bronchiolitis defined by length of hospital stay, supportive care, clinical score and parental visual analogue scale and early asthma development. (paper #2, 4)

Among the 294 infants from the bronchiolitis group who attended the 2-year follow-up study, 49% had recurrent wheeze at two years of age, while 19% had physician-diagnosed asthma. We found no significant association between bronchiolitis severity and early asthma development, reported as recurrent wheeze at two years of age. Bronchiolitis severity was assessed in several ways, using the physician-recorded clinical score, parental VAS scores at hospitalization, LOS and use of supportive care during hospital stay. The infants receiving supportive care (50%) had a significant longer hospital stay compared to those not receiving supportive care (114 hours vs 45 hours, $p<0.001$).

Our findings that severity of acute bronchiolitis was not associated with subsequent recurrent wheeze are in contrast to previous studies reporting a strong relationship between bronchiolitis severity and subsequent early asthma development (132-135). However, these latter studies defined severity of bronchiolitis as the need for hospital admission. A Dutch study describe a 3.1-fold increased risk of asthma development among infants hospitalised with RSV bronchiolitis compared to non-hospitalised children (136). In our study, all infants had moderate to severe acute bronchiolitis leading to hospitalisation as well as a clinical score of at least 4 out of 10. Thus, our finding that more severe disease within the spectrum of hospitalised infants did not increase the risk of later asthma development must be interpreted within this limitation.

In our study, 49% of the infants hospitalised with acute bronchiolitis who attended the two-year follow-up visit had recurrent wheeze at two years of age, while 19% had physician diagnosed asthma. This is similar to a Finnish study (134) in which 50% of infants hospitalised with bronchiolitis had asthma at 4 years of age. On the other hand, a Swedish study (132) found that 23% of the patients with RSV bronchiolitis had current asthma at 7 years of age among infants hospitalised with RSV bronchiolitis in line with two studies from the United States describing 24% and 28% of the children hospitalised with bronchiolitis being diagnosed with asthma at 4 and 5 years of age, respectively

(133, 135). First, our study cannot out rule an overestimation of recurrent wheeze, as there may be an overrepresentation of children with ongoing respiratory disease among the children who attended the 2-year visit, compared to infants lost to follow-up. Second, asthma at the age of 7 is not the same as recurrent wheeze at 2 years of age, knowing that asthma diagnosis is more questionable at younger age.

In the study by Sigurs and co-workers (78), the increased risk of asthma persisted into early adulthood. Among the 47 children hospitalised with RSV bronchiolitis <1 year of age, 18 (39%) were diagnosed with asthma at 18 years of age (78).

Without a control group we were unable to determine the specific excess rate of asthma due to acute bronchiolitis, while our results indicate that receiving supportive care did not further increase the risk of asthma beyond being hospitalised for acute bronchiolitis.

5.4 The prevalence of early allergic sensitisation and the role of allergic sensitisation, type or load of viruses or salivary cortisol during acute bronchiolitis in infancy for asthma development

We found that 8.5% of the infants in our study populations were sensitised to at least one allergen, food being the most common, observed in 7.4% of the individuals, with sensitisation to inhalant allergens in 1.9%. Our finding of allergic sensitisation in 8.5% of the study population is lower than the 12.5% at 3 months and 16.7% at 6 months in the DARC- study (86), but in line with the $COPSAC_{2000}$ high- risk cohort (83) reporting that 7.8% of six- months old infants were sensitised (IgE ≥0.35 kU/l) to food allergens and 0.6% to inhalant allergens. With no significant differences in allergic sensitisation between the bronchiolitis group and control groups, we were able to show that around 5% of infants 0-3 months were sensitised, almost all to food allergens, with rates around 10% in infants older than > 3 months. Our results are in line with a recent population-based randomised clinical trial on early complementary feeding recently reporting allergic sensitisation based on SPT of at least 1 mm in 5.3% of 3-month old infants, while 2.9% had a SPT to food allergens of at least 5 mm (137). As expected, we found that infants older than compared to younger than four months were significantly more often sensitised to allergens.

Allergic sensitisation in infants younger than 12 months was not significantly associated with recurrent wheeze at two years of age. To the best of our knowledge, ours is the first study to assess a potential role of allergic sensitisation in very young infants for early asthma development. Although allergic sensitisation is a well-known risk factor for asthma development (88, 90, 138-141) and allergic disease in general (86), in contrast to our study population with an overall mean age of 5.1 months (142), most studies assessed allergic sensitisation from 18 months to 8 years of age. The only exception is DARC study (86) with allergic sensitisation assessed by sIgE and SPT first year of life at 0, 3, 6 and 12 months. Sly and co-workers (143) postulated the importance of early recognition of allergic sensitisation to identify children at risk of developing asthma. On the other hand, a Finnish

study found that among children with the first episode of wheeze from 3-23 months of age, allergic sensitisation increased the risk of asthma at 8 years of age (144). Most of these children were also hospitalised but at a mean age of 11 months, and 17% sensitised to any allergen. The observation time of two years in our study may have been too short a period to observe asthma developing later in school age.

Our finding that there was no association between type of virus at acute bronchiolitis and recurrent wheeze at two years of age is supported by other studies (6, 90, 145). In an Australian birth cohort study (90) there was no difference in recurrent wheeze at five years of age comparing those with HRV and RSV associated wheeze in infancy. Moreover, a Swedish and Norwegian study found that asthma risk in early childhood was independent of the presence of RSV during acute infant bronchiolitis (6, 145). This suggests that the risk of persistent wheeze and asthma development after viral bronchiolitis is independent of specific respiratory pathogens. In our cohort, RSV were found in 53%, HRV in 6% and both viruses in 29% of the infants. In comparison, an Italian study (146) found RSV in 43%, HRV in 9% and both viruses in 0.4%, and a Finnish study (147) found RSV in 63%, HRV in 11%, and both viruses in 3% when hospitalised with acute bronchiolitis. Both studies found HRV bronchiolitis to be most associated with recurrent wheeze in early childhood.

In our study high viral load was associated with more severe bronchiolitis (16), in line with other studies (28), whereas viral load was not associated with early asthma development. To our knowledge, this is the first study that has investigated this association.

Whether viral bronchiolitis in infancy or early childhood promotes asthma development or identifies those infants at risk for subsequent wheezing is not known. During infancy there is a rapid growth and development of the pulmonary and immune system, and viral infection alters the subsequent pattern of the Th1/Th2 immune response (148). Both respiratory viruses inducing a direct cytotoxic injury to the airways and the host inflammatory response contribute to the bronchiolitis pathogenesis (12) and to the severity of the bronchiolitis (148). Not all children with bronchiolitis will

develop asthma, which also might suggest a role of virus- and/ or host-specific factors in the asthma pathogenesis (149). It is further hypothesised that RSV infection is more severe in infants with some underlying predispositions (76), which per se contributes to later bronchial obstructive disease (132).

Our observation that there were no interactions between RSV and HRV bronchiolitis in infancy and allergic sensitisation for the risk of early asthma development, to the best of our knowledge, is novel. Our results do not support previous studies that found allergic sensitisation potentiated the increased risk of asthma conferred by HRV LRTI (90, 91). An Australian study, including newborns with atopic parents, showed that HRV lower respiratory tract infection in the first year of life contributed to persistent wheeze and asthma at 5 years of age in children who were sensitised by 2 years of age, defined by a SPT with wheal size ≥2 mm (90). In the COAST study, allergic sensitisation to aeroallergens at one year of age, defined by IgE ≥ 0.35 kU/l, was found to predispose children to HRV wheezing illness (91). The lack of significant associations between allergic sensitisation and early asthma development may be related to the very young age of sensitisation, mostly to food allergens in our study, in contrast to aeroallergen sensitisation being more common in the DARC study (86) Furthermore, our end-point of recurrent wheeze at two years of age may be more heterogenous than asthma observed in pre-school or school age in the other studies (90, 132, 144).

The lack of significant association between cortisol level in infants hospitalised with acute bronchiolitis and recurrent wheeze at two years of age, to our knowledge, has not been described previously. We have shown in this study that children with acute bronchiolitis in infancy had higher levels of morning salivary cortisol than controls at enrolment, but with similar levels at two years of age (100). The higher level of cortisol during this bronchiolitis is likely to reflect an acute stress response. It has been claimed that stress plays an important role in the development of chronic asthma (150). In animal models, short-term stress increasing the corticosteroid level results in a reduction of inflammatory cell infiltration in the airways, while long-term stress, not involving the

corticosteroids, causes an increase in airway inflammatory cells and an increased airway hyperresponsiveness (150). Children with asthma have been found to have lower baseline serum cortisol (151) and salivary cortisol (103) levels compared with healthy controls. Increased levels of salivary cortisol during acute infant bronchiolitis does not appear to be associated with recurrent wheeze by two years of age, and it is still unclear to what extent cortisol plays a role in asthma development.

The thesis aimed to answer the role of allergic sensitisation, type or load of viruses or salivary cortisol during acute bronchiolitis in infancy for asthma development, identifying other risk factors were outside the scope of the present thesis.

Our findings suggest that asthma development has no significant association to allergic sensitisation in infancy, specific viruses or viral load during as well as salivary cortisol, as a marker of stress during acute bronchiolitis. Identification of those infants at risk of developing early asthma, with or without acute bronchiolitis hospitalisation, may provide important knowledge about the different asthma phenotypes, asthma treatment and eventually the prevention of the asthmatic disease.

5.5 Strengths and limitations

5.5.1 Strengths

The multicentre prospective design including the majority of hospitals in south-eastern Norway strengthened the generalisability of the study. The study was conducted around the clock, in the real-life clinical practice involving a large number of nurses and physicians, circumstances that subsequently strengthened the generalisability of our findings.

The prospective design with a thorough follow-up by the study team and principal investigators ensured complete data set for all short-term and prognostic outcomes.

The fact that physician-conducted structured interviews were held at study inclusion and two-year follow-up instead of gathering data through self-reporting questionnaires may have improved the accuracy of the reported background characteristics.

The infants underwent multiple assessments related to severity of clinical presentation, including several scores and objective registrations in parallel. The parental evaluation of bronchiolitis severity in the Bronchiolitis ALL study is novel. Parental evaluation by VAS provided important knowledge about the value of involving parents in clinical decision-making.

Our detailed virus diagnostics, including a high number of viruses and viral load, allergic sensitisation analyses and salivary cortisol sampling in infancy yielded novel insight and added further knowledge of bronchiolitis disease and long-term prognosis.

The control group from the general population was a strength in terms of understanding and generalising allergic sensitisation in infancy in general.

Recurrent wheeze was defined as three or more episodes of bronchial obstruction, used as a proxy for early asthma development in this thesis. In view of the challenges of diagnosing asthma at this early age, our outcome is strengthened by the fact that more than 80% of the children with recurrent wheeze reported having used asthma medication, compared to only 20% without recurrent wheeze.

5.5.2 Limitations

Infants with chronic lung disease, congenital heart disease, neuromuscular disorders, immunodeficiency and other serious chronic diseases may have a more severe course of bronchiolitis (31). However, serious pre-existing diseases could not be assessed as risk factors in our study, as infants with serious disease that may influence the results of the interventions in the RCT were excluded from the bronchiolitis ALL study.

Length of hospital stay was defined as the time from hospital admission to the actual time of discharge. This decision was made by the physician on duty, depending on disease severity, use of supportive care, parental social factors and the distance to home, which together with, a crowded ward in some cases, might have influenced the hospital discharge.

The clinical score used in our study was not previously validated, albeit reported in another Scandinavian study investigating the effect of inhaled nebulized racemic adrenaline upon symptoms of acute bronchiolitis (42). However, the score is in line with other clinical scores combining objective and subjective parameters used to predict hospital admission (32) and to evaluate the efficacy of treatment for acute wheezing in infants (39).

Parental assessment may have been influenced by health care workers prior to presenting to hospital? However, the parents were asked to score their child by the VAS score as soon as possible after study enrolment, in order to avoid this possible bias.

Although the loss to follow-up at two years of age was 27%, apart from higher education attainment, there were no differences in background characteristics comparing those attending and those did not attend the two-year follow-up. Thus, the potential impact of loss to follow-up may have resulted in bias affecting the validity of the inferences drawn from the study.

The overall low number of children with allergic sensitisation at study enrolment may limit the statistical power to detect significant interaction with HRV or other viruses for asthma development,

a type 2- error. However, the study populations included in this thesis are in size comparable to similar studies elsewhere.

The approximately two-month age difference between the bronchiolitis group and the control group may have influenced the reported incidence of atopic sensitisation, given that the risk of allergic sensitization increases with increasing age.

6 Implications of the study

The assessment at hospital admission by parental VAS predicted the use of supportive care during hospital stay. Assessing the severity of illness in a child may be generally challenging. The highly variable course of the bronchiolitis further challenges treatment management. Including parental judgement in a structural evaluation may improve bronchiolitis management.

A Clinical score and SpO_2–measurement, although useful in clinical decision making to some extent, have limitations, as shown by the rather limited sensitivity and specificity for receiving supportive care. Our results suggest that most factors at hospitalization related to receiving supportive care still remains unclear.

The Bronchiolitis study has contributed significantly to international guidelines in terms of defining the role of inhaled therapy during acute bronchiolitis. The finding in our study that inhalations of racemic adrenaline was not superior to inhalations of isotonic saline have contributed towards improving national and international guidelines on the management of acute bronchiolitis. No inhalation strategies have so far proven to decrease disease severity and LOS. Infants receiving inhalations on demand were hospitalised for significantly shorter periods of time and received significantly less supportive care compared to infants treated with inhalation on a fixed schedule. This novel and important knowledge has emphasised that inhalation therapy during acute bronchiolitis may not be the right choice of treatment. Healthcare providers should only provide inhalation therapy that leads to observed benefits for the patients, and supports the recommendation that every handling procedure should be questioned and if necessary kept as simple and short as possible (152).

Although we found no significant association between allergic sensitization in infancy and recurrent wheeze at two years, there is still a large knowledge gap in understanding the role of early allergic sensitization in infancy, especially in relation to the atopic march and further asthma development.

The identification of specific viruses during bronchiolitis disease could have been an important guide to disease severity and to identification of children at greater risk of developing asthma. The lack of associations between RSV and HRV and disease severity and early asthma development in our study questions the need for specific viral diagnostics during acute bronchiolitis. On the other hand, viral diagnostics may guide the decision making of abstaining from antibiotic therapy. Our finding that infants with RSV high genomic load more often received supportive care and had a longer LOS, may encourage more use of cluster analyses in clinical practice and further studies of the impact of viral load.

7 Main conclusions

To identify risk factors for receiving supportive care for acute moderate to severe infant bronchiolitis. (paper #1, 2)

Caesarion section delivery, $SpO_2 < 92\%$, increased heart rate and lower age at hospitalisation were found to increase the risk for receiving supportive care during hospital stay.

To determine if clinical score or parental assessment using a visual analogue scale of acute infant bronchiolitis at the time of hospitalisation predicts the short-term prognosis. (paper #1, 2)

The clinical score and the three parental VAS modalities at hospital admission predicted the use of supportive care and LOS, with the exception of the VAS item feeding being associated with supportive care only.

To determine if severity of acute bronchiolitis, defined by length of hospital stay, receiving supportive care, clinical score or parental visual analogue scale is associated with early asthma development. (paper #2, 4)

We found no significant association between the severity of acute bronchiolitis among infants hospitalised for acute bronchiolitis and early asthma development.

To determine prevalence of early allergic sensitization and the role of allergic sensitisation, type or load of viruses or salivary cortisol during acute bronchiolitis in infancy for asthma development. (paper # 2, 3, 4)

Allergic sensitisation, specific viruses and viral load or salivary morning cortisol did not increase the risk of early asthma development in children hospitalised with acute bronchiolitis during first year of life.

8 Future perspectives

No inhalation therapies are proven to decrease disease severity and LOS. Our study showed that getting inhalations on demand were associated with reduced LOS. A recent published systemic review suggest that nebulised normal saline could be an active treatment for acute bronchiolitis (153). Future studies should focus on the effect of inhalation therapy overall versus no treatment. Is inhalation therapy more beneficial for certain subgroups?

Since the Bronchiolitis ALL study, there has been a greater focus on optimising respiratory support (154), and nasal high flow therapy has been introduced (155). Nasal high flow gives a high inspiratory flow of heated and humidified gas consisting of a mixture of oxygen and air (154). Studies guiding the use in paediatric wards are lacking (154, 155). In general, future trials should focus on how to best assess and manage the supportive care in infants hospitalised with acute bronchiolitis.

Parental involvement in clinical decision making is emphasised. Future studies in bronchiolitis or other paediatric illnesses should include parental assessment, collaboration or a view to exploring how best to include parental involvement in the decision making.

Considering all studies published addressing the treatment, management and later risk of asthma development, bronchiolitis is not a homogenous disease. Some studies have shown that only infants with allergic sensitisation have developed persistent wheeze after bronchiolitis while wheezing has diminished in non-sensitised children. Future studies and clinical trials should try to identify bronchiolitis phenotypes and eventually link them to wheezing or asthma phenotypes.

9 References

1. Court SD. The definition of acute respiratory illnesses in children. Postgraduate medical journal. 1973;49(577):771-6.

2. Leader S, Kohlhase K. Recent trends in severe respiratory syncytial virus (RSV) among US infants, 1997 to 2000. The Journal of pediatrics. 2003;143(5 Suppl):S127-32.

3. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The burden of respiratory syncytial virus infection in young children. The New England journal of medicine. 2009;360(6):588-98.

4. Wright AL, Taussig LM, Ray CG, Harrison HR, Holberg CJ. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. American journal of epidemiology. 1989;129(6):1232-46.

5. Carlsen KH, Orstavik I, Halvorsen K. Viral infections of the respiratory tract in hospitalized children. A study from Oslo during a 90 months' period. Acta Paediatr Scand. 1983;72(1):53-8.

6. Fjaerli HO, Farstad T, Rod G, Ufert GK, Gulbrandsen P, Nakstad B. Acute bronchiolitis in infancy as risk factor for wheezing and reduced pulmonary function by seven years in Akershus County, Norway. BMC pediatrics. 2005;5:31.

7. Jartti T, Makela MJ, Vanto T, Ruuskanen O. The link between bronchiolitis and asthma. Infect Dis Clin North Am. 2005;19(3):667-89.

8. American Academy of Pediatrics Subcommittee on D, Management of B. Diagnosis and management of bronchiolitis. Pediatrics. 2006;118(4):1774-93.

9. Bont L, Checchia PA, Fauroux B, Figueras-Aloy J, Manzoni P, Paes B, et al. Defining the Epidemiology and Burden of Severe Respiratory Syncytial Virus Infection Among Infants and Children in Western Countries. Infect Dis Ther. 2016;5(3):271-98.

10. Carroll KN, Gebretsadik T, Griffin MR, Wu P, Dupont WD, Mitchel EF, et al. Increasing burden and risk factors for bronchiolitis-related medical visits in infants enrolled in a state health care insurance plan. Pediatrics. 2008;122(1):58-64.

11. Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. Respiratory syncytial virus-associated hospitalizations among infants and young children in the United States, 1997-2006. The Pediatric infectious disease journal. 2012;31(1):5-9.

12. Meissner HC. Viral Bronchiolitis in Children. The New England journal of medicine. 2016;374(1):62-72.

13. Dawson K, Kennedy D, Asher I, Cooper D, Cooper P, Francis P, et al. The management of acute bronchiolitis. Thoracic Society of Australia and New Zealand. Journal of paediatrics and child health. 1993;29(5):335-7.

14. Meissner HC. Viral Bronchiolitis in Children. The New England journal of medicine. 2016;374(18):1793-4.

15. Smyth RL, Openshaw PJ. Bronchiolitis. Lancet. 2006;368(9532):312-22.

16. Skjerven HO, Megremis S, Papadopoulos NG, Mowinckel P, Carlsen KH, Lodrup Carlsen KC, et al. Virus Type and Genomic Load in Acute Bronchiolitis: Severity and Treatment Response With Inhaled Adrenaline. J Infect Dis. 2016;213(6):915-21.

17. Mansbach JM, Piedra PA, Teach SJ, Sullivan AF, Forgey T, Clark S, et al. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. Archives of pediatrics & adolescent medicine. 2012;166(8):700-6.

18. Jartti T, Smits HH, Bonnelykke K, Bircan O, Elenius V, Konradsen JR, et al. Bronchiolitis needs a revisit: Distinguishing between virus entities and their treatments. Allergy. 2019;74(1):40-52.

19. Turunen R, Koistinen A, Vuorinen T, Arku B, Soderlund-Venermo M, Ruuskanen O, et al. The first wheezing episode: respiratory virus etiology, atopic characteristics, and illness severity. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2014;25(8):796-803.

20. Jartti T, Lehtinen P, Vuorinen T, Ruuskanen O. Bronchiolitis: age and previous wheezing episodes are linked to viral etiology and atopic characteristics. The Pediatric infectious disease journal. 2009;28(4):311-7.

21. Wainwright C. Acute viral bronchiolitis in children- a very common condition with few therapeutic options. Paediatric respiratory reviews. 2010;11(1):39-45; quiz

22. Hall CB. Respiratory syncytial virus and parainfluenza virus. The New England journal of medicine. 2001;344(25):1917-28.

23. Hall CB, Weinberg GA, Blumkin AK, Edwards KM, Staat MA, Schultz AF, et al. Respiratory syncytial virus-associated hospitalizations among children less than 24 months of age. Pediatrics. 2013;132(2):e341-8.

24. Koehoorn M, Karr CJ, Demers PA, Lencar C, Tamburic L, Brauer M. Descriptive epidemiological features of bronchiolitis in a population-based cohort. Pediatrics. 2008;122(6):1196-203.

25. Kneyber MCJ, Steyerberg EW, de Groot R, Moll HA. Long-term effects of respiratory syncytial virus (RSV) bronchiolitis in infants and young children: a quantitative review. Acta Paediatr. 2000;89(6):654-60.

26. Brand HK, de Groot R, Galama JM, Brouwer ML, Teuwen K, Hermans PW, et al. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. Pediatric pulmonology. 2012;47(4):393-400.

27. Jartti T, Aakula M, Mansbach JM, Piedra PA, Bergroth E, Koponen P, et al. Hospital length-of-stay is associated with rhinovirus etiology of bronchiolitis. The Pediatric infectious disease journal. 2014;33(8):829-34.

28. DeVincenzo JP, El Saleeby CM, Bush AJ. Respiratory syncytial virus load predicts disease severity in previously healthy infants. The Journal of infectious diseases. 2005;191(11):1861-8.

29. Jartti T, Hasegawa K, Mansbach JM, Piedra PA, Camargo CA, Jr. Rhinovirus-induced bronchiolitis: Lack of association between virus genomic load and short-term outcomes. J Allergy Clin Immunol. 2015;136(2):509-12 e11.

30. Hunderi JOG, Rolfsjord LB, Carlsen KCL, Holst R, Bakkeheim E, Berents TL, et al. Virus, allergic sensitisation and cortisol in infant bronchiolitis and risk of early asthma. ERJ Open Res. 2020;6(1).

31. Ricci V, Delgado Nunes V, Murphy MS, Cunningham S, Guideline Development G, Technical T. Bronchiolitis in children: summary of NICE guidance. BMJ. 2015;350:h2305.

32. Walsh P, Rothenberg SJ, O'Doherty S, Hoey H, Healy R. A validated clinical model to predict the need for admission and length of stay in children with acute bronchiolitis. European journal of emergency medicine : official journal of the European Society for Emergency Medicine. 2004;11(5):265-72.

33. Donlan M, Fontela PS, Puligandla PS. Use of continuous positive airway pressure (CPAP) in acute viral bronchiolitis: a systematic review. Pediatric pulmonology. 2011;46(8):736-46.

34. Willwerth BM, Harper MB, Greenes DS. Identifying hospitalized infants who have bronchiolitis and are at high risk for apnea. Annals of emergency medicine. 2006;48(4):441-7.

35. Chamberlain JM, Patel KM, Pollack MM. Association of emergency department care factors with admission and discharge decisions for pediatric patients. The Journal of pediatrics. 2006;149(5):644-9.

36. Chamberlain JM, Patel KM, Ruttimann UE, Pollack MM. Pediatric risk of admission (PRISA): a measure of severity of illness for assessing the risk of hospitalization from the emergency department. Annals of emergency medicine. 1998;32(2):161-9.

37. Duncan H, Hutchison J, Parshuram CS. The Pediatric Early Warning System score: a severity of illness score to predict urgent medical need in hospitalized children. Journal of critical care. 2006;21(3):271-8.

38. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Critical care medicine. 1996;24(5):743-52.

39. Tal A, Bavilski C, Yohai D, Bearman JE, Gorodischer R, Moses SW. Dexamethasone and salbutamol in the treatment of acute wheezing in infants. Pediatrics. 1983;71(1):13-8.

40. Lowell DI, Lister G, Von Koss H, McCarthy P. Wheezing in infants: the response to epinephrine. Pediatrics. 1987;79(6):939-45.

41. Wang EE, Milner RA, Navas L, Maj H. Observer agreement for respiratory signs and oximetry in infants hospitalized with lower respiratory infections. The American review of respiratory disease. 1992;145(1):106-9.

42. Kristjansson S, Lodrup Carlsen KC, Wennergren G, Strannegard IL, Carlsen KH. Nebulised racemic adrenaline in the treatment of acute bronchiolitis in infants and toddlers. Archives of disease in childhood. 1993;69(6):650-4.

43. Voets S, van Berlaer G, Hachimi-Idrissi S. Clinical predictors of the severity of bronchiolitis. European journal of emergency medicine : official journal of the European Society for Emergency Medicine. 2006;13(3):134-8.

44. Corneli HM, Zorc JJ, Holubkov R, Bregstein JS, Brown KM, Mahajan P, et al. Bronchiolitis: clinical characteristics associated with hospitalization and length of stay. Pediatric emergency care. 2012;28(2):99-103.

45. Marlais M, Evans J, Abrahamson E. Clinical predictors of admission in infants with acute bronchiolitis. Archives of disease in childhood. 2011;96(7):648-52.

46. Bekhof J, Reimink R, Brand PL. Systematic review: insufficient validation of clinical scores for the assessment of acute dyspnoea in wheezing children. Paediatric respiratory reviews. 2014;15(1):98-112.

47. Van den Bruel A, Haj-Hassan T, Thompson M, Buntinx F, Mant D, European Research Network on Recognising Serious Infection i. Diagnostic value of clinical features at presentation to identify serious infection in children in developed countries: a systematic review. Lancet. 2010;375(9717):834-45.

48. Lipstein EA, Brinkman WB, Britto MT. What is known about parents' treatment decisions? A narrative review of pediatric decision making. Med Decis Making. 2012;32(2):246-58.

49. Rodriguez-Martinez CE, Sossa-Briceno MP, Nino G. Systematic review of instruments aimed at evaluating the severity of bronchiolitis. Paediatric respiratory reviews. 2018;25:43-57.

50. McCallum GB, Morris PS, Wilson CC, Versteegh LA, Ward LM, Chatfield MD, et al. Severity scoring systems: are they internally valid, reliable and predictive of oxygen use in children with acute bronchiolitis? Pediatric pulmonology. 2013;48(8):797-803.

51. De Boeck K, Van der Aa N, Van Lierde S, Corbeel L, Eeckels R. Respiratory syncytial virus bronchiolitis: a double-blind dexamethasone efficacy study. The Journal of pediatrics. 1997;131(6):919-21.

52. Destino L, Weisgerber MC, Soung P, Bakalarski D, Yan K, Rehborg R, et al. Validity of respiratory scores in bronchiolitis. Hosp Pediatr. 2012;2(4):202-9.

53. De Brasi D, Pannuti F, Antonelli F, de Seta F, Siani P, de Seta L. Therapeutic approach to bronchiolitis: why pediatricians continue to overprescribe drugs? Ital J Pediatr. 2010;36:67.

54. Chin HJ, Seng QB. Reliability and validity of the respiratory score in the assessment of acute bronchiolitis. Malays J Med Sci. 2004;11(2):34-40.

55. Beck R, Elias N, Shoval S, Tov N, Talmon G, Godfrey S, et al. Computerized acoustic assessment of treatment efficacy of nebulized epinephrine and albuterol in RSV bronchiolitis. BMC pediatrics. 2007;7:22.

56. Wainwright C, Altamirano L, Cheney M, Cheney J, Barber S, Price D, et al. A multicenter, randomized, double-blind, controlled trial of nebulized epinephrine in infants with acute bronchiolitis. The New England journal of medicine. 2003;349(1):27-35.

57. Gajdos V, Beydon N, Bommenel L, Pellegrino B, de Pontual L, Bailleux S, et al. Inter-observer agreement between physicians, nurses, and respiratory therapists for respiratory clinical evaluation in bronchiolitis. Pediatric pulmonology. 2009;44(8):754-62.

58. Liu LL, Gallaher MM, Davis RL, Rutter CM, Lewis TC, Marcuse EK. Use of a respiratory clinical score among different providers. Pediatric pulmonology. 2004;37(3):243-8.

59. Mecklin M, Hesselmar B, Qvist E, Wennergren G, Korppi M. Diagnosis and treatment of bronchiolitis in Finnish and Swedish children's hospitals. Acta Paediatr. 2014;103(9):946-50.

60. Vandini S, Faldella G, Lanari M. Latest options for treatment of bronchiolitis in infants. Expert Rev Respir Med. 2016;10(4):453-61.

61. Parikh K, Hall M, Teach SJ. Bronchiolitis management before and after the AAP guidelines. Pediatrics. 2014;133(1):e1-7.

62. M.H. P, Carlsen KC. Akuttt Bronkiolitt. Veileder i akutt akutt pediatri. 2006.

63. Wohl ME, Chernick V. State of the art: bronchiolitis. The American review of respiratory disease. 1978;118(4):759-81.

64. Zhang L, Sanguebsche LS. [The safety of nebulization with 3 to 5 ml of adrenaline (1:1000) in children: an evidence based review]. J Pediatr (Rio J). 2005;81(3):193-7.

65. Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE, Gadomski AM, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics. 2014;134(5):e1474-502.

66. Skjerven HO, Hunderi JO, Brugmann-Pieper SK, Brun AC, Engen H, Eskedal L, et al. Racemic adrenaline and inhalation strategies in acute bronchiolitis. The New England journal of medicine. 2013;368(24):2286-93.

67. Pettersen MH, Carlsen KC. Akutt bronkiolitt. Veileder i akutt akutt pediatri Den Norske legeforening, Oslo, norway. 2007:198-201.

68. Reijonen T, Korppi M, Pitkakangas S, Tenhola S, Remes K. The clinical efficacy of nebulized racemic epinephrine and albuterol in acute bronchiolitis. Archives of pediatrics & adolescent medicine. 1995;149(6):686-92.

69. Sanchez I, De Koster J, Powell RE, Wolstein R, Chernick V. Effect of racemic epinephrine and salbutamol on clinical score and pulmonary mechanics in infants with bronchiolitis. The Journal of pediatrics. 1993;122(1):145-51.

70. Lodrup Carlsen KC, Carlsen KH. Inhaled nebulized adrenaline improves lung function in infants with acute bronchiolitis. Respiratory medicine. 2000;94(7):709-14.

71. Langley JM, Smith MB, LeBlanc JC, Joudrey H, Ojah CR, Pianosi P. Racemic epinephrine compared to salbutamol in hospitalized young children with bronchiolitis; a randomized controlled clinical trial [ISRCTN46561076]. BMC pediatrics. 2005;5(1):7.

72. Hariprakash S, Alexander J, Carroll W, Ramesh P, Randell T, Turnbull F, et al. Randomized controlled trial of nebulized adrenaline in acute bronchiolitis. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2003;14(2):134-9.

73. Patel H, Platt RW, Pekeles GS, Ducharme FM. A randomized, controlled trial of the effectiveness of nebulized therapy with epinephrine compared with albuterol and saline in infants hospitalized for acute viral bronchiolitis. The Journal of pediatrics. 2002;141(6):818-24.

74. Kirolos A, Manti S, Blacow R, Tse G, Wilson T, Lister M, et al. A Systematic Review of Clinical Practice Guidelines for the Diagnosis and Management of Bronchiolitis. The Journal of infectious diseases. 2019.

75. Jartti T, Gern JE. Role of viral infections in the development and exacerbation of asthma in children. J Allergy Clin Immunol. 2017;140(4):895-906.

76. Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. The New England journal of medicine. 1988;319(17):1112-7.

77. Lemanske RF, Jr., Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol. 2005;116(3):571-7.

78. Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. Thorax. 2010;65(12):1045-52.

79. Nilsson SF, Lilja G, Jarnbert-Pettersson H, Alm J. Relevance of low specific IgE levels to egg, milk and peanut in infancy. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2019;49(3):308-16.

80. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test - European standards. Clin Transl Allergy. 2013;3(1):3.

81. Bernstein IL, Li JT, Bernstein DI, Hamilton R, Spector SL, Tan R, et al. Allergy diagnostic testing: an updated practice parameter. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2008;100(3 Suppl 3):S1-148.

82. Alduraywish SA, Lodge CJ, Vicendese D, Lowe AJ, Erbas B, Matheson MC, et al. Sensitization to milk, egg and peanut from birth to 18 years: A longitudinal study of a cohort at risk of allergic disease. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2016;27(1):83-91.

83. Schoos AM, Chawes BL, Folsgaard NV, Samandari N, Bonnelykke K, Bisgaard H. Disagreement between skin prick test and specific IgE in young children. Allergy. 2015;70(1):41-8.

84. Schoos AM, Chawes BL, Rasmussen MA, Bloch J, Bonnelykke K, Bisgaard H. Atopic endotype in childhood. J Allergy Clin Immunol. 2016;137(3):844-51 e4.

85. Bousquet J, Anto JM, Wickman M, Keil T, Valenta R, Haahtela T, et al. Are allergic multimorbidities and IgE polysensitization associated with the persistence or re-occurrence of foetal type 2 signalling? The MeDALL hypothesis. Allergy. 2015;70(9):1062-78.

86. Kjaer HF, Eller E, Andersen KE, Host A, Bindslev-Jensen C. The association between early sensitization patterns and subsequent allergic disease. The DARC birth cohort study. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2009;20(8):726-34.

87. Alduraywish SA, Lodge CJ, Campbell B, Allen KJ, Erbas B, Lowe AJ, et al. The march from early life food sensitization to allergic disease: a systematic review and meta-analyses of birth cohort studies. Allergy. 2016;71(1):77-89.

88. Almqvist C, Li Q, Britton WJ, Kemp AS, Xuan W, Tovey ER, et al. Early predictors for developing allergic disease and asthma: examining separate steps in the 'allergic march'. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2007;37(9):1296-302.

89. Holt PG, Upham JW, Sly PD. Contemporaneous maturation of immunologic and respiratory functions during early childhood: implications for development of asthma prevention strategies. J Allergy Clin Immunol. 2005;116(1):16-24; quiz 5.

90. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol. 2007;119(5):1105-10.

91. Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, Lee WM, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. American journal of respiratory and critical care medicine. 2012;185(3):281-5.

92. Asthma GIf. Asthma management and prevention. Global Initiative for Asthma. Irish medical journal. Updated 2019;Suppl:i-vi, 1-39.

93. Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology. 2008;32(4):1096-110.

94. Pedersen SE, Hurd SS, Lemanske RF, Jr., Becker A, Zar HJ, Sly PD, et al. Global strategy for the diagnosis and management of asthma in children 5 years and younger. Pediatric pulmonology. 2011;46(1):1-17.

95. Brand PL, Caudri D, Eber E, Gaillard EA, Garcia-Marcos L, Hedlin G, et al. Classification and pharmacological treatment of preschool wheezing: changes since 2008. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology. 2014;43(4):1172-7.

96. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. The New England journal of medicine. 1995;332(3):133-8.

97. Young S, O'Keeffe PT, Arnott J, Landau LI. Lung function, airway responsiveness, and respiratory symptoms before and after bronchiolitis. Archives of disease in childhood. 1995;72(1):16-24.

98. Stevenson EC, Turner G, Heaney LG, Schock BC, Taylor R, Gallagher T, et al. Bronchoalveolar lavage findings suggest two different forms of childhood asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1997;27(9):1027-35.

99. Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. Journal of psychosomatic research. 2002;53(4):865-71.

100. Rolfsjord LB, Bakkeheim E, Berents TL, Alm J, Skjerven HO, Carlsen KH, et al. Morning Salivary Cortisol in Young Children: Reference Values and the Effects of Age, Sex, and Acute Bronchiolitis. J Pediatr. 2017;184:193-8 e3.

101. Pinto RA, Arredondo SM, Bono MR, Gaggero AA, Diaz PV. T helper 1/T helper 2 cytokine imbalance in respiratory syncytial virus infection is associated with increased endogenous plasma cortisol. Pediatrics. 2006;117(5):e878-86.

102. Priftis KN, Papadimitriou A, Nicolaidou P, Chrousos GP. Dysregulation of the stress response in asthmatic children. Allergy. 2009;64(1):18-31.

103. Bakkeheim E, Mowinckel P, Carlsen KH, Burney P, Lodrup Carlsen KC. Reduced basal salivary cortisol in children with asthma and allergic rhinitis. Acta Paediatr. 2010;99(11):1705-11.

104. Forsythe P, Ebeling C, Gordon JR, Befus AD, Vliagoftis H. Opposing effects of short- and long-term stress on airway inflammation. American journal of respiratory and critical care medicine. 2004;169(2):220-6.

105. Tryphonopoulos PD, Letourneau N, Azar R. Approaches to salivary cortisol collection and analysis in infants. Biol Res Nurs. 2014;16(4):398-408.

106. Oakley E, Bata S, Rengasamy S, Krieser D, Cheek J, Jachno K, et al. Nasogastric Hydration in Infants with Bronchiolitis Less Than 2 Months of Age. The Journal of pediatrics. 2016;178:241-5 e1.

107. Oymar K, Bardsen K. Continuous positive airway pressure for bronchiolitis in a general paediatric ward; a feasibility study. BMC pediatrics. 2014;14:122.

108. Mansbach JM, Piedra PA, Stevenson MD, Sullivan AF, Forgey TF, Clark S, et al. Prospective multicenter study of children with bronchiolitis requiring mechanical ventilation. Pediatrics. 2012;130(3):e492-500.

109. Papoff P, Moretti C, Cangiano G, Bonci E, Roggini M, Pierangeli A, et al. Incidence and predisposing factors for severe disease in previously healthy term infants experiencing their first episode of bronchiolitis. Acta Paediatr. 2011;100(7):e17-23.

110. Hendaus MA, Jomha FA, Alhammadi AH. Pulse oximetry in bronchiolitis: is it needed? Ther Clin Risk Manag. 2015;11:1573-8.

111. Neff TA. Routine oximetry. A fifth vital sign? Chest. 1988;94(2):227.

112. Mower WR, Sachs C, Nicklin EL, Baraff LJ. Pulse oximetry as a fifth pediatric vital sign. Pediatrics. 1997;99(5):681-6.

113. Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980-1996. Jama. 1999;282(15):1440-6.

114. Mallory MD, Shay DK, Garrett J, Bordley WC. Bronchiolitis management preferences and the influence of pulse oximetry and respiratory rate on the decision to admit. Pediatrics. 2003;111(1):e45-51.

115. Shay DK, Holman RC, Roosevelt GE, Clarke MJ, Anderson LJ. Bronchiolitis-associated mortality and estimates of respiratory syncytial virus-associated deaths among US children, 1979-1997. The Journal of infectious diseases. 2001;183(1):16-22.

116. British Thoracic Society Standards of Care C. British Thoracic Society Guidelines for the Management of Community Acquired Pneumonia in Childhood. Thorax. 2002;57 Suppl 1:i1-24.

117. Elliott M, Tate R, Page K. Do clinicians know how to use pulse oximetry? A literature review and clinical implications. Australian critical care : official journal of the Confederation of Australian Critical Care Nurses. 2006;19(4):139-44.

118. Sala KA, Moore A, Desai S, Welch K, Bhandari S, Carroll CL. Factors associated with disease severity in children with bronchiolitis. The Journal of asthma : official journal of the Association for the Care of Asthma. 2015;52(3):268-72.

119. Stevens TP, Sinkin RA, Hall CB, Maniscalco WM, McConnochie KM. Respiratory syncytial virus and premature infants born at 32 weeks' gestation or earlier: hospitalization and economic implications of prophylaxis. Archives of pediatrics & adolescent medicine. 2000;154(1):55-61.

120. Buckingham SC, Bush AJ, Devincenzo JP. Nasal quantity of respiratory syncytical virus correlates with disease severity in hospitalized infants. The Pediatric infectious disease journal. 2000;19(2):113-7.

121. Gorelick MH. Severity of illness measures for pediatric emergency care: are we there yet? Annals of emergency medicine. 2003;41(5):639-43.

122. Streiner DL. A checklist for evaluating the usefulness of rating scales. Can J Psychiatry. 1993;38(2):140-8.

123. Dawson KP. The severity of acute asthma attacks in children admitted to hospital. Australian paediatric journal. 1987;23(3):167-8.

124. Wennergren G, Engstrom I, Bjure J. Transcutaneous oxygen and carbon dioxide levels and a clinical symptom scale for monitoring the acute asthmatic state in infants and young children. Acta Paediatr Scand. 1986;75(3):465-9.

125. Gorelick MH, Stevens MW, Schultz TR, Scribano PV. Performance of a novel clinical score, the Pediatric Asthma Severity Score (PASS), in the evaluation of acute asthma. Academic emergency medicine : official journal of the Society for Academic Emergency Medicine. 2004;11(1):10-8.

126. Gadomski AM, Lichenstein R, Horton L, King J, Keane V, Permutt T. Efficacy of albuterol in the management of bronchiolitis. Pediatrics. 1994;93(6 Pt 1):907-12.

127. Bekhof J, Reimink R, Bartels IM, Eggink H, Brand PL. Large observer variation of clinical assessment of dyspnoeic wheezing children. Archives of disease in childhood. 2015;100(7):649-53.

128. Mansbach JM, Clark S, Christopher NC, LoVecchio F, Kunz S, Acholonu U, et al. Prospective multicenter study of bronchiolitis: predicting safe discharges from the emergency department. Pediatrics. 2008;121(4):680-8.

129. Shepperd S, Perera R, Bates S, Jenkinson C, Hood K, Harnden A, et al. A children's acute respiratory illness scale (CARIFS) predicted functional severity and family burden. Journal of clinical epidemiology. 2004;57(8):809-14.

130. MacFaul R, Stewart M, Werneke U, Taylor-Meek J, Smith HE, Smith IJ. Parental and professional perception of need for emergency admission to hospital: prospective questionnaire based study. Archives of disease in childhood. 1998;79(3):213-8.

131. Gorelick MH, Stevens MW, Schultz TR. Comparability of acute asthma severity assessments by parents and respiratory therapists. Archives of pediatrics & adolescent medicine. 2002;156(12):1199-202.

132. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. American journal of respiratory and critical care medicine. 2000;161(5):1501-7.

133. Carroll KN, Wu P, Gebretsadik T, Griffin MR, Dupont WD, Mitchel EF, et al. The severity-dependent relationship of infant bronchiolitis on the risk and morbidity of early childhood asthma. J Allergy Clin Immunol. 2009;123(5):1055-61, 61 e1.

134. Reijonen TM, Kotaniemi-Syrjanen A, Korhonen K, Korppi M. Predictors of asthma three years after hospital admission for wheezing in infancy. Pediatrics. 2000;106(6):1406-12.

135. Balekian DS, Linnemann RW, Hasegawa K, Thadhani R, Camargo CA, Jr. Cohort Study of Severe Bronchiolitis during Infancy and Risk of Asthma by Age 5 Years. J Allergy Clin Immunol Pract. 2017;5(1):92-6.

136. Zomer-Kooijker K, van der Ent CK, Ermers MJ, Uiterwaal CS, Rovers MM, Bont LJ, et al. Increased risk of wheeze and decreased lung function after respiratory syncytial virus infection. PloS one. 2014;9(1):e87162.

137. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. The New England journal of medicine. 2016;374(18):1733-43.

138. Sherrill D, Stein R, Kurzius-Spencer M, Martinez F. On early sensitization to allergens and development of respiratory symptoms. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1999;29(7):905-11.

139. Peat JK, Salome CM, Woolcock AJ. Longitudinal changes in atopy during a 4-year period: relation to bronchial hyperresponsiveness and respiratory symptoms in a population sample of Australian schoolchildren. J Allergy Clin Immunol. 1990;85(1 Pt 1):65-74.

140. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U, et al. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet. 2006;368(9537):763-70.

141. Rhodes HL, Thomas P, Sporik R, Holgate ST, Cogswell JJ. A birth cohort study of subjects at risk of atopy: twenty-two-year follow-up of wheeze and atopic status. American journal of respiratory and critical care medicine. 2002;165(2):176-80.

142. Skjerven HO, Gjengsto Hunderi JO, Carlsen KH, Rolfsjord LB, Nordhagen L, Berents TL, et al. Allergic sensitisation in infants younger than one year of age. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2019.

143. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, et al. Early identification of atopy in the prediction of persistent asthma in children. Lancet. 2008;372(9643):1100-6.

144. Lukkarinen M, Koistinen A, Turunen R, Lehtinen P, Vuorinen T, Jartti T. Rhinovirus-induced first wheezing episode predicts atopic but not nonatopic asthma at school age. J Allergy Clin Immunol. 2017;140(4):988-95.

145. Wennergren G, Hansson S, Engstrom I, Jodal U, Amark M, Brolin I, et al. Characteristics and prognosis of hospital-treated obstructive bronchitis in children aged less than two years. Acta Paediatr. 1992;81(1):40-5.

146. Midulla F, Nicolai A, Ferrara M, Gentile F, Pierangeli A, Bonci E, et al. Recurrent wheezing 36 months after bronchiolitis is associated with rhinovirus infections and blood eosinophilia. Acta Paediatr. 2014;103(10):1094-9.

147. Bergroth E, Aakula M, Korppi M, Remes S, Kivisto JE, Piedra PA, et al. Post-bronchiolitis Use of Asthma Medication: A Prospective 1-year Follow-up Study. The Pediatric infectious disease journal. 2016;35(4):363-8.

148. Singh AM, Moore PE, Gern JE, Lemanske RF, Jr., Hartert TV. Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. American journal of respiratory and critical care medicine. 2007;175(2):108-19.

149. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. American journal of respiratory and critical care medicine. 2008;178(7):667-72.

150. Vig RS, Forsythe P, Vliagoftis H. The role of stress in asthma: insight from studies on the effect of acute and chronic stressors in models of airway inflammation. Annals of the New York Academy of Sciences. 2006;1088:65-77.

151. Landstra AM, Postma DS, Boezen HM, van Aalderen WM. Role of serum cortisol levels in children with asthma. American journal of respiratory and critical care medicine. 2002;165(5):708-12.

152. Murdoch DR, Darlow BA. Handling during neonatal intensive care. Archives of disease in childhood. 1984;59(10):957-61.

153. House SA, Gadomski AM, Ralston SL. Evaluating the Placebo Status of Nebulized Normal Saline in Patients With Acute Viral Bronchiolitis: A Systematic Review and Meta-analysis. JAMA Pediatr. 2020.

154. Franklin D, Fraser JF, Schibler A. Respiratory support for infants with bronchiolitis, a narrative review of the literature. Paediatric respiratory reviews. 2019;30:16-24.

155. Lin J, Zhang Y, Xiong L, Liu S, Gong C, Dai J. High-flow nasal cannula therapy for children with bronchiolitis: a systematic review and meta-analysis. Archives of disease in childhood. 2019;104(6):564-76.

I

ORIGINAL ARTICLE

Racemic Adrenaline and Inhalation Strategies in Acute Bronchiolitis

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ABSTRACT

BACKGROUND

Acute bronchiolitis in infants frequently results in hospitalization, but there is no established consensus on inhalation therapy — either the type of medication or the frequency of administration — that may be of value. We aimed to assess the effectiveness of inhaled racemic adrenaline as compared with inhaled saline and the strategy for frequency of inhalation (on demand vs. fixed schedule) in infants hospitalized with acute bronchiolitis.

METHODS

In this eight-center, randomized, double-blind trial with a 2-by-2 factorial design, we compared inhaled racemic adrenaline with inhaled saline and on-demand inhalation with fixed-schedule inhalation (up to every 2 hours) in infants (<12 months of age) with moderate-to-severe acute bronchiolitis. An overall clinical score of 4 or higher (on a scale of 0 to 10, with higher scores indicating more severe illness) was required for study inclusion. Any use of oxygen therapy, nasogastric-tube feeding, or ventilatory support was recorded. The primary outcome was the length of the hospital stay, with analyses conducted according to the intention-to-treat principle.

RESULTS

The mean age of the 404 infants included in the study was 4.2 months, and 59.4% were boys. Length of stay, use of oxygen supplementation, nasogastric-tube feeding, ventilatory support, and relative improvement in the clinical score from baseline (preinhalation) were similar in the infants treated with inhaled racemic adrenaline and those treated with inhaled saline (P>0.1 for all comparisons). On-demand inhalation, as compared with fixed-schedule inhalation, was associated with a significantly shorter estimated mean length of stay — 47.6 hours (95% confidence interval [CI], 30.6 to 64.6) versus 61.3 hours (95% CI, 45.4 to 77.2; P=0.01) — as well as less use of oxygen supplementation (in 38.3% of infants vs. 48.7%, P=0.04), less use of ventilatory support (in 4.0% vs. 10.8%, P=0.01), and fewer inhalation treatments (12.0 vs. 17.0, P<0.001).

CONCLUSIONS

In the treatment of acute bronchiolitis in infants, inhaled racemic adrenaline is not more effective than inhaled saline. However, the strategy of inhalation on demand appears to be superior to that of inhalation on a fixed schedule. (Funded by Medicines for Children; ClinicalTrials.gov number, NCT00817466; EudraCT number, 2009-012667-34.)

Acute bronchiolitis in infants, which frequently leads to hospitalization[1,2] and sometimes requires ventilatory support, is occasionally fatal[3]; it is usually viral in origin, with respiratory syncytial virus[4] being the most common cause. The clinical disease is characterized by nasal flaring, tachypnea, dyspnea, chest retractions, crepitations, and wheezing.[5]

Bronchodilators are not recommended[6,7] but are often used in the treatment of bronchiolitis,[8-10] as are saline inhalations. Adrenaline reduces mucosal swelling,[11] giving it an edge over the β_2-adrenergic agonists,[12] and has led to the frequent use of inhaled adrenaline,[13] which has improved symptoms[12,14-20] and reduced the need for hospitalization in outpatients with acute bronchiolitis.[12] Among inpatients, however, inhaled adrenaline has not been found to reduce the length of the hospital stay.[12,20-22] Assessment of the possible influences of age, sex, and status with respect to an asthma predisposition[23] on the effect of inhaled adrenaline requires large multicenter studies.[12,24]

Inhaled nebulized solutions can be prescribed for use on demand or on a fixed schedule. We were unable to find documentation on the comparative efficacy of these two strategies in children with acute bronchiolitis.

We tested the hypothesis that inhaled racemic adrenaline is superior to inhaled saline in the treatment of acute bronchiolitis in infancy and that administration on a fixed schedule is superior to administration on demand. We also assessed whether age, sex, or status with respect to allergic diseases influenced treatment efficacy.

Methods

Study Design

This multicenter, double-blind, randomized clinical trial (the Bronchiolitis All-study, SE-Norway) included infants with acute bronchiolitis who were admitted to the pediatric departments of eight hospitals in southeastern Norway from January 2010 through May 2011. In accordance with a 2-by-2 factorial design, children were randomly assigned to receive inhaled racemic adrenaline or inhaled saline and to receive the assigned treatment on demand or on a fixed schedule (Fig. 1).

The study was approved by the Regional Committees for Medical and Health Research Ethics and by the Norwegian Medicines Agency and is registered in the Norwegian Biobank Registry. Written informed consent was obtained from a parent of each child before the start of therapy. The study was audited by the Norwegian Medicines Agency in 2011. All authors vouch for the accuracy and completeness of the reported data and for the fidelity of the report to the study protocol (available with the full text of this article at NEJM.org).

The inclusion criteria were clinical signs of bronchiolitis as defined by Court[5] (see the Supplementary Appendix, available at NEJM.org), an age of less than 12 months, and an overall clinical score of at least 4 on a scale of 0 to 10. The clinical score was the sum of points allotted, from 0 (indicating normal findings) to 2 (indicating severe illness), for each of the following: general condition, skin color, findings on auscultation, respiratory rate, and retractions[15,25] (Table S1 in the Supplementary Appendix). The study physicians performing the clinical scoring were trained at investigator meetings as well as on site by the first author and by local primary investigators. The exclusion criteria were the presence of any serious cardiac, immunologic, neurologic, or oncologic disease or any serious pulmonary disease other than bronchiolitis; more than one previous episode of obstructive airway disease; symptoms of disease of the lower airway (e.g., coughing) for more than 4 weeks; and receipt of any glucocorticoid therapy in the preceding 4 weeks.

Children were enrolled in the study on admission to the hospital as long as attending personnel (a physician and a nurse) were available. Clinical scoring was performed by a pediatrician. After written informed consent was obtained from a parent, children underwent randomization, and the assigned study medication was administered. The baseline characteristics of the children were obtained on admission, and the assessment included a pediatrician-guided, structured interview of one or both parents. Viral analyses of nasopharyngeal aspirates were performed at the largest hospital involved in the study (Oslo University Hospital) with the use of a polymerase-chain-reaction assay for nine common airway viruses. (See the Supplementary Appendix for further information on the biologic specimens gathered.)

Randomization was performed centrally in blocks of eight, with assignment to one of the four study groups, with the use of SAS software, version 9.3. The randomization codes were com-

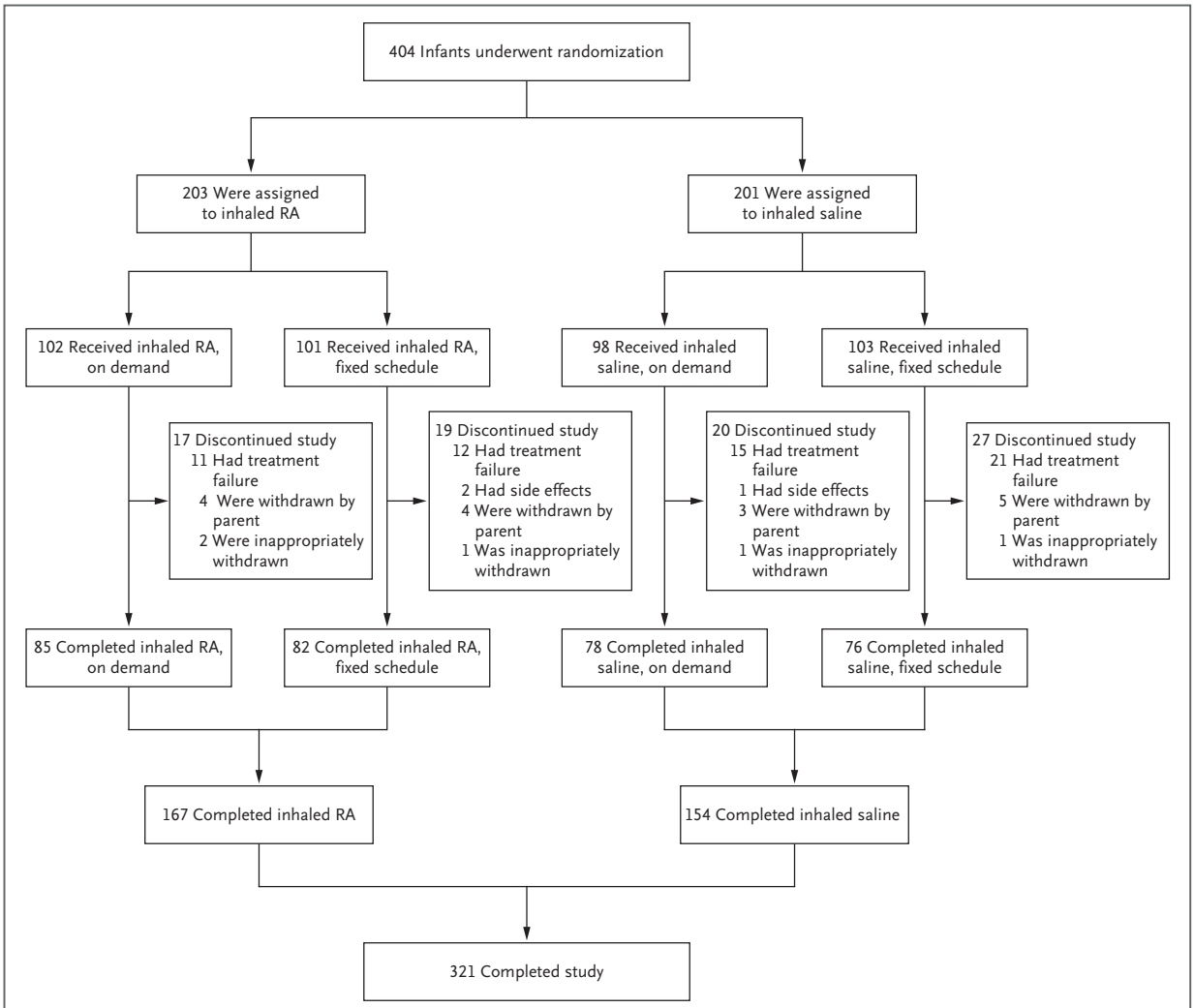

Figure 1. Randomization of the Study Patients.

In five children, the study medication was discontinued because of the following administrative failures: administration of open inhaled racemic adrenaline (RA), suspected pertussis infection, delayed biologic sampling, administration of a dose of the study medication that was too high, and insufficient supply of study medication.

municated directly by the study statistician to the pharmacy, where doses of the two study medications (10 ml of racemic adrenaline dissolved in 0.9% saline to form a solution of 20 mg per milliliter or 0.9% saline alone) were prepared in identical bottles, each labeled with a numerical code indicating the type of medication and timing of administration (on demand or fixed schedule). The study centers, which were not aware of the randomization block size, were provided with a list of study numbers for use in the consecutive assignment of medication to enrolled children.

The dose administered was based on the infant's weight: 0.10 ml for infants weighing less than 5 kg, 0.15 ml for those weighing 5 to 6.9 kg, 0.20 ml for those weighing 7 to 9.9 kg, and 0.25 ml for those weighing 10 kg or more.[15] The medications were diluted in 2 ml of saline before nebulization and were administered through a Sidestream Reusable Nebulizer with a Respironics Facemask (both from Philips Respironics), driven by 100% oxygen at a rate of 6 liters per minute. No other inhaled medications, with the exception of 0.9% inhaled saline (which was an option in both study groups, to be administered

at the discretion of the attending physician), could be administered during the period when the infant was participating in the trial. Supportive therapy and any other treatments were provided in accordance with routine care. In accordance with national guidelines, glucocorticoids and β_2-adrenergic agonists were not administered.[13]

OUTCOMES

The primary outcome, length of hospital stay, was defined as the time from the first study inhalation until discharge from the hospital, as recorded in the medical record for each patient. Secondary outcomes were the change in the clinical score 30 minutes after the first inhalation and the use of nasogastric-tube feeding, oxygen supplementation, or ventilatory support, all of which were recorded throughout the patient's hospital stay. Adverse events during hospitalization were monitored and reported within 24 hours.

Clinical scores, oxygen saturation as measured by pulse oximetry, heart rate, respiratory rate, the use of nasogastric-tube feeding, the use of ventilatory support, and the time at which each inhalation occurred were recorded from one to four times daily during hospitalization (see the Supplementary Appendix for details). Treatment with supplemental oxygen and the performance of chest radiography were also recorded.

STATISTICAL ANALYSES

Continuous data are presented as means (±SD), and categorical data are presented as numbers and percentages. Categorical data were assessed with the use of the Pearson chi-square test. Because data on length of stay had a non-normal distribution, comparisons between groups were assessed with the use of a robust, two-sample t-test and Huber's M-estimator, with 95% confidence intervals.

Interactions were assessed for inhaled racemic adrenaline versus inhaled saline and on-demand versus fixed-schedule administration, as well as for treatment and site, with the use of robust linear regression and Huber's M-estimator. The Jonckheere–Terpstra test was used to assess interactions between age (at 3-month intervals) and interventions. Local regression smoothing was applied to assess the effect of age on length of stay.

The power analysis was based on the length of stay of approximately 450 children hospitalized at the main study site during a 12-month period before the start of the study. Assuming that clinically relevant improvement would be indicated by a length of stay that was reduced by at least 5 hours in the group receiving inhaled racemic adrenaline,[26] we calculated that a total of 176 children in each medication group would provide a power of at least 80% at a two-sided alpha level of 0.05. Owing to the inclusion of secondary outcomes and subgroup analyses, we increased the enrollment target to a total of 500 children. The level of significance was set at 0.05, and analyses were performed with the use of SAS software, version 9.3, and IBM SPSS software, version 19.

RESULTS

STUDY PATIENTS

The study included 404 children (59.4% of whom were boys) with a mean age of 126 days (4.2 months) (Table 1). The number of children enrolled at each study center ranged from 22 to 136 (mean, 51) (Table S2 in the Supplementary Appendix). The study medication was discontinued in 83 children (20.5%) for the reasons listed in Figure 1.

The mean (±SD) length of stay for all infants was 80±67 hours; most children were discharged between 8 a.m. and 11 p.m. (Fig. S1 in the Supplementary Appendix). Baseline characteristics did not differ significantly among the four study groups (Table 1).

Routine respiratory viral assays were performed in 123 of the 136 children admitted to Oslo University Hospital; 99 of those 123 children (80.5%) were positive for respiratory syncytial virus and 21 of 123 children (17.1%) were positive for another virus; 5 of 123 (4.1%) children were positive for two viruses.

RACEMIC ADRENALINE VERSUS SALINE

There was no significant difference in length of hospital stay between children treated with inhaled racemic adrenaline and those treated with inhaled saline (P=0.43) (Table 2 and Fig. 2A). There were also no significant between-group differences in the use of nasogastric-tube feeding, supplemental oxygen, or ventilatory support; clinical scores before and after the first inhalation of the study medication; or the number of children in whom the study medication was discontinued (36 children in the group receiving inhaled racemic adrenaline and 47 in the group receiving inhaled saline) (Table 2).

Table 1. Baseline Characteristics of the Study Patients.*

Characteristics	Inhaled Racemic Adrenaline		Inhaled Saline	
	On Demand (N=102)	Fixed Schedule (N=101)	On Demand (N=98)	Fixed Schedule (N=103)
Male sex — no. (%)	63 (61.8)	60 (59.4)	54 (55.1)	63 (61.2)
Mean age — days	134.9±91.6	116.9±87.8	117.8±68.1	136.0±97.0
Parental race — no./total no. (%)†				
Father white	79/87 (90.8)	85/90 (94.4)	75/83 (90.4)	83/91 (91.2)
Mother white	79/88 (89.8)	85/92 (92.4)	78/84 (92.9)	83/92 (90.2)
Medical history — no./total no. (%)				
Atopic eczema	12/92 (13.0)	8/96 (8.3)	6/90 (6.6)	14/96 (14.6)
Allergies	4/87 (4.6)	0/96 (0)	1/90 (1.1)	2/96 (2.1)
1 previous wheeze	24/88 (27.3)	23/91 (25.3)	20/90 (22.2)	31/93 (33.3)
Respiratory symptoms for >1 wk — no./total no. (%)	8/75 (10.7)	12/90 (13.3)	10/86 (11.6)	15/89 (16.9)
Parental medical history — no./total no. (%)				
Asthma	17/78 (21.8)	22/83 (26.5)	23/80 (28.8)	21/84 (25.0)
Rhinoconjunctivitis	23/88 (26.1)	33/89 (37.1)	23/87 (26.4)	34/92 (37.0)
Clinical characteristics before study inclusion				
Clinical score‡	4.9±1.0	5.0±1.0	4.9±1.0	4.9±1.0
SpO_2§	96.0±3.6	96.0±3.3	96.0±3.4	96.1±2.8
Respiratory rate — breaths/min	53.1±11.8	53.6±10.5	53.8±11.3	53.4±11.1
Heart rate — beats/min	154.5±17.5	156.0±18.7	155.2±19.9	153.7±17.7

* Plus–minus values are means ±SD. No significant differences in baseline characteristics were found among the four groups.
† Race was determined by the investigator.
‡ A clinical score of 4 or higher (on a range of 0 to 10, with 0 being the best score) was required for study inclusion.
§ SpO_2 denotes oxygen saturation as measured by pulse oximetry.

ON-DEMAND VERSUS FIXED-SCHEDULE ADMINISTRATION

The mean length of the hospital stay was significantly shorter for children in the group receiving treatment on demand than in the group receiving treatment on a fixed schedule (P=0.01) (Table 2 and Fig. 2B). Children in the on-demand group received a mean of 5.0 (30%) fewer inhalations than those in the fixed-schedule group (P<0.001). Children receiving inhalations on demand also had a lower probability of being treated with ventilatory support (P=0.01) or supplemental oxygen (P=0.04), and inhalations given on demand were not associated with nasogastric-tube feeding or treatment discontinuation (Table 2).

There was no interaction between the two treatment interventions (inhaled racemic adrenaline vs. inhaled saline and on-demand vs. fixed schedule), with an estimated interaction term of 1.4 hours (95% confidence interval [CI], –20.1 to 22.8; P=0.90) (Table S4 in the Supplementary Appendix.)

INFLUENCE OF AGE, ALLERGIC DISEASE, AND SEX

Age (in 3-month periods) had a significant effect on length of hospital stay with regard to both medication type and inhalation strategy, as estimated with the use of the Jonckheere–Terpstra test (P<0.001). The stay was longer in the younger infants (<8 weeks of age) than in the older infants (≥8 weeks of age) in the group receiving inhaled racemic adrenaline group, as indicated with a curve showing the median at every time point (Fig. S2A and S2B in the Supplementary Appendix).

In subgroup analyses comparing children younger than 3 months of age (177, or 43.8% of the study population) with those 3 months of age or older, there was no significant difference between the effect of inhaled racemic adrenaline

Table 2. Length of Stay and Use of Supportive Therapy According to Medication and Inhalation Strategy.

Variable	Inhaled Racemic Adrenaline (N=203)	Inhaled Saline (N=201)	Difference or Rate Ratio (95% CI)*	P Value	On Demand (N=200)	Fixed Schedule (N=204)	Difference or Rate Ratio (95% CI)*	P Value
Length of stay — hr†								
Mean	78.7	81.8			73.9	86.5		
Range	69.2 to 88.1	72.6 to 91.0			64.6 to 83.2	77.1 to 95.8		
Estimated length of stay — hr†								
Mean	63.6	68.1			47.6	61.3		
Range	46.2 to 81.0	49.8 to 86.4			30.6 to 64.6	45.4 to 77.2		
Mean difference			4.5 (−6.5 to 15.5)	0.42			13.7 (2.9 to 24.4)	0.01
Change in clinical score after 1 inhalation‡								
Mean	−1.26	−1.08			−1.18	−1.16		
Range	−1.44 to −1.08	−1.23 to −0.92			−1.35 to −1.02	−1.33 to −0.98		
No. of inhalations								
Mean	13.9	15.2			12.0	17.0		
Range	12.1 to 15.7	13.2 to 17.2			10.3 to 13.6	15.0 to 19.1		
Supportive therapy — no./total no. (%)								
Oxygen	83/192 (43.2)	83/189 (43.9)	0.98 (0.78 to 1.24)		72/188 (38.3)	94/193 (48.7)	0.79 (0.62 to 0.99)	0.04
Nasogastric-tube feeding	57/201 (28.4)	59/199 (29.6)	0.96 (0.70 to 1.30)		52/198 (26.3)	64/202 (31.7)	0.83 (0.61 to 1.13)	
Ventilatory support	15/203 (7.4)	15/201 (7.5)	0.99 (0.50 to 1.97)		8/200 (4.0)	22/204 (10.8)	0.37 (0.17 to 0.81)	0.01
Discontinued treatment — no./total no. (%)	36/203 (17.7)	47/201 (23.4)	0.76 (0.52 to 1.12)		37/200 (18.5)	46/204 (22.5)	0.82 (0.56 to 1.21)	

* Rate ratios are shown for supportive therapy.
† The mean given for "Length of stay" was an unweighted mean. The mean given for "Estimated length of stay" was a weighted mean estimated with the use of robust linear regression analysis.
‡ There were 377 children with clinical scoring before and after the first inhalation at the time of study enrollment.

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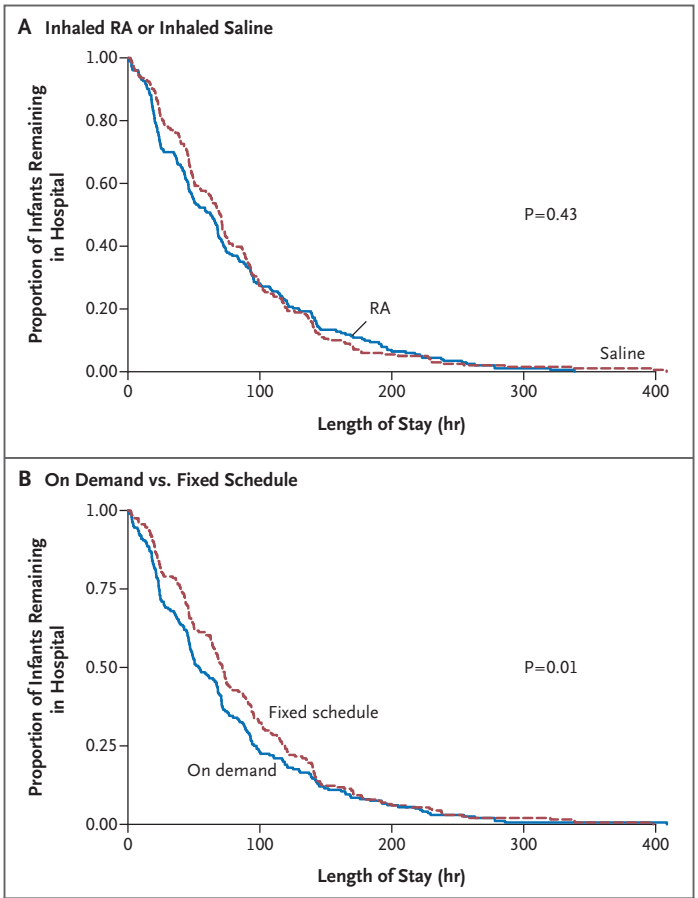

Figure 2. Length of Hospital Stay According to Study Treatment and Schedule of Administration.

Kaplan–Meier plots show the proportion of infants remaining in the hospital according to whether they were treated with inhaled racemic adrenaline or inhaled saline (Panel A) and according to whether the inhalations were administered on demand or on a fixed schedule (Panel B).

as compared with that of inhaled saline. In the youngest children only, inhalations given on demand were associated with a significantly shorter hospital stay than were inhalations given on a fixed schedule (Table S3 in the Supplementary Appendix). Status with respect to a history of atopic eczema or wheezing, status with respect to a family history of atopic disease, and sex were not found to have a significant influence on treatment response.

ADVERSE EVENTS

No serious adverse events were reported. Three children (including one who was receiving inhaled saline) discontinued treatment because of moderate tachycardia, which may have been due to the study medication.

DISCUSSION

In infants with acute bronchiolitis, treatment with inhalations of racemic adrenaline was not associated with a shorter hospital stay than treatment with inhaled saline. However, the administration of inhalations on demand was found to be superior to administration on a fixed schedule in reducing the length of stay and in reducing the use of ventilatory support, supplemental oxygen therapy, and nasogastric-tube feeding. There was an interaction between age and either medication type or inhalation strategy.

The lack of effect of inhaled racemic adrenaline on length of hospital stay confirms similar findings on length of stay for albuterol and saline[21] and for albuterol alone.[22] There was a similar lack of effect of these medications on the clinical score and oxygen saturation according to a Cochrane meta-analysis,[12] including the findings in 292 patients from two trials[20,21] reviewed in the meta-analysis.

Our data show that inhalations given on demand are superior to those administered on a fixed schedule in children younger than 12 months of age, with the mean length of stay 13.7 hours shorter for those receiving inhalations on demand. This difference was both clinically and statistically significant and has substantial financial implications. Although not previously shown, the possibility that saline may have a bronchoconstrictive effect in the youngest infants (younger than 3 months of age) cannot be ruled out. Thus, the superiority of the on-demand schedule, in which fewer inhalations were administered, supports the goal of "minimal handling" (allowing infants to sleep, with minimal interruption)[27] in acutely ill infants.

The significant interaction we noted between age and inhaled racemic adrenaline is of interest. The tendency for the youngest infants receiving inhaled racemic adrenaline to have a longer hospital stay does not support the reported effectiveness of inhaled racemic adrenaline for reducing vascular engorgement and edema in children with asthma.[11] Also unlike the findings in children with asthma,[28] in our study population of children with bronchiolitis, status with respect to a parental history of allergic disease, status with respect to atopic eczema, and sex were not associated with symptomatic treatment efficacy.

Our study of two inhalation solutions was sufficiently powered to allow detection of a

5-hour difference in length of stay and to perform subgroup analyses for the major outcomes. The study included a nationally representative patient cohort with the expected patterns of viral infection.[4] In addition, the study was managed in accordance with local and national guidelines, and the baseline characteristics were similar in all four treatment groups (Table S1 in the Supplementary Appendix).

Despite the limited power of the study to detect an interaction between the interventions, the observed interaction was approximately one third of the 5-hour length of stay selected a priori as a clinically relevant difference.[26] The initially planned end point for length of stay, which was the time at which the child was deemed ready for discharge, was not recorded for 83 children (Fig. 1). We therefore used the actual time of discharge for all children. The results were similar with the use of these two end points (Table S5 in the Supplementary Appendix).

In conclusion, our study showed that for hospitalized infants with acute bronchiolitis, inhaled racemic adrenaline was not superior to inhaled saline with regard to length of hospital stay, use of supportive treatment, or clinical score. However, the administration of inhalations on demand, as compared with a fixed schedule of inhalations, was associated with a shorter hospital stay and with a reduced need for supportive treatment.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the nurses and pediatricians at all the participating hospitals for their invaluable contribution to this project.

REFERENCES

1. Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. Respiratory syncytial virus-associated hospitalizations among infants and young children in the United States, 1997-2006. Pediatr Infect Dis J 2012;31:5-9.
2. Leader S, Kohlhase K. Respiratory syncytial virus-coded pediatric hospitalizations, 1997 to 1999. Pediatr Infect Dis J 2002;21:629-32.
3. Donlan M, Fontela PS, Puligandla PS. Use of continuous positive airway pressure (CPAP) in acute viral bronchiolitis: a systematic review. Pediatr Pulmonol 2011;46:736-46.
4. Mansbach JM, McAdam AJ, Clark S, et al. Prospective multicenter study of the viral etiology of bronchiolitis in the emergency department. Acad Emerg Med 2008; 15:111-8.
5. Court SD. The definition of acute respiratory illnesses in children. Postgrad Med J 1973;49:771-6.
6. American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis. Diagnosis and management of bronchiolitis. Pediatrics 2006; 118:1774-93.
7. Baumer JH. SIGN guideline on bronchiolitis in infants. Arch Dis Child Educ Pract Ed 2007;92(5):ep149-ep151.
8. Law BJ, De Carvalho V. Respiratory syncytial virus infections in hospitalized Canadian children: regional differences in patient populations and management practices. Pediatr Infect Dis J 1993;12:659-63.
9. Newcomb RW. Use of adrenergic bronchodilators by pediatric allergists and pulmonologists. Am J Dis Child 1989;143: 481-5.
10. Kimpen JL, Schaad UB. Treatment of respiratory syncytial virus bronchiolitis: 1995 poll of members of the European Society for Paediatric Infectious Diseases. Pediatr Infect Dis J 1997;16:479-81.
11. Wohl ME, Chernick V. State of the art: bronchiolitis. Am Rev Respir Dis 1978;118: 759-81.
12. Hartling L, Bialy LM, Vandermeer B, et al. Epinephrine for bronchiolitis. Cochrane Database Syst Rev 2011;6:CD003123.
13. Pettersen MH, Halvorsen T, Carlsen KH. Akutt bronkiolitt. Veileder i akutt pediatri. Oslo: Den norske lægeforening, 2007:198-201. (In Norwegian.)
14. Hariprakash S, Alexander J, Carroll W, et al. Randomized controlled trial of nebulized adrenaline in acute bronchiolitis. Pediatr Allergy Immunol 2003;14:134-9.
15. Kristjánsson S, Lødrup Carlsen KC, Wennergren G, Strannegard IL, Carlsen KH. Nebulised racemic adrenaline in the treatment of acute bronchiolitis in infants and toddlers. Arch Dis Child 1993;69:650-4.
16. Lødrup Carlsen KC, Carlsen KH. Inhaled nebulized adrenaline improves lung function in infants with acute bronchiolitis. Respir Med 2000;94:709-14.
17. Menon K, Sutcliffe T, Klassen TP. A randomized trial comparing the efficacy of epinephrine with salbutamol in the treatment of acute bronchiolitis. J Pediatr 1995; 126:1004-7.
18. Reijonen T, Korppi M, Pitkäkangas S, Tenhola S, Remes K. The clinical efficacy of nebulized racemic epinephrine and albuterol in acute bronchiolitis. Arch Pediatr Adolesc Med 1995;149:686-92.
19. Sanchez I, De Koster J, Powell RE, Wolstein R, Chernick V. Effect of racemic epinephrine and salbutamol on clinical score and pulmonary mechanics in infants with bronchiolitis. J Pediatr 1993; 122:145-51.
20. Wainwright C, Altamirano L, Cheney M, et al. A multicenter, randomized, double-blind, controlled trial of nebulized epinephrine in infants with acute bronchiolitis. N Engl J Med 2003;349:27-35.
21. Patel H, Platt RW, Pekeles GS, Ducharme FM. A randomized, controlled trial of the effectiveness of nebulized therapy with epinephrine compared with albuterol and saline in infants hospitalized for acute viral bronchiolitis. J Pediatr 2002;141:818-24.
22. Langley JM, Smith MB, LeBlanc JC, Joudrey H, Ojah CR, Pianosi P. Racemic epinephrine compared to salbutamol in hospitalized young children with bronchiolitis; a randomized controlled clinical trial. BMC Pediatr 2005;5:7.
23. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med 2005;171:137-41.
24. Smyth RL, Openshaw PJ. Bronchiolitis. Lancet 2006;368:312-22.
25. Wennergren G, Engström I, Bjure J. Transcutaneous oxygen and carbon dioxide levels and a clinical symptom scale for monitoring the acute asthmatic state in infants and young children. Acta Paediatr Scand 1986;75:465-9.
26. Panickar J, Lakhanpaul M, Lambert PC, et al. Oral prednisolone for preschool children with acute virus-induced wheezing. N Engl J Med 2009;360:329-38.
27. Murdoch DR, Darlow BA. Handling during neonatal intensive care. Arch Dis Child 1984;59:957-61.
28. Bacharier LB, Boner A, Carlsen KH, et al. Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. Allergy 2008;63:5-34. [Erratum, Allergy 2008;63:630.]

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Skjerven HO, Hunderi JOG, Brügmann-Pieper SK, et al. Racemic adrenaline and inhalation strategies in acute bronchiolitis. N Engl J Med 2013;368:2286-93. DOI: 10.1056/NEJMoa1301839

Supplementary Appendix

Racemic Adrenaline and Inhalation Strategies in Acute Bronchiolitis

(Bronchiolitis All-study SE Norway, ClincialTrials.gov number, NCT00817466. EudraCT number, 2009-012667-34)

A study performed within the ORAACLE, Oslo, Norway

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The study was performed within ORAACLE (the Oslo Research Group of Asthma and Allergy in Childhood; the Lung and Environment), a member of GA^{2}LEN (Global Asthma and Allergy European Network) and MeDALL (Mechanisms of the Development of ALLergy) a collaborative project conducted within the European Union under the Health Cooperation Work Programme of the 7th Framework programme (grant agreement No. 261357)

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Introduction

Length of stay in bronchiolitis is mostly influenced by supportive treatment [1] such as nasogastric feeding and oxygen supplementation and less commonly by ventilatory support[2,3]

Methods

Clinical scoring

Acute bronchiolitis was defined by Court as following:

"Illness mainly affecting infants, especially in the first 6 months of life. Rapid respiration, dyspnea, wheezing, chest recession, cough, rhonchi and rales are very frequent. Visible distension of the chest and increased pulmonary translucency on the chest radiograph are frequent and of high diagnostic significance. Upper respiratory features, especially nasal discharge and a red pharynx are frequent. Fever is very frequent, but high fever is uncommon."

Very frequent is noted as at least 50% of the children, while frequent refers to 25-50%.

Clinical registrations and management

The clinical score (Table S1) was recorded by a physician prior to and 30 minutes after an inhalation every morning. Parents and the attending nurse each completed a visual analogue scale (VAS) and the nurse noted oxygen saturation, respiratory rates and heart rates prior to and 30 minutes after the first inhalation and subsequently once every morning and evening. Saliva was sampled upon inclusion and in the first morning, and nasopharyngeal aspirates and urinary samples were collected as soon as possible after inclusion. All stored biological samples were frozen within 24 hours for further analysis. Biological samples, nurse and parental VAS scale as well as clinical scoring by physician throughout the hospital stay will not be further discussed in the present paper.

Registration of the use of nasogastric tube feeding and ventilatory support and time for each inhalation were recorded daily.

Results

Influence of age

In children younger than three months only, inhalations given OD compared to RI was associated with a relative risk of 0.37 (0.15-0.90) of patients receiving treatment with ventilatory support (p=0.021). (Table S3)

Discussion

The clinical scoring (Table S1) used in the present study had previously been used in a Scandinavian study[4], but was, in line with other scoring systems of acute bronchiolitis, not validated[5]. The commonly used Respiratory Distress Assessment Instrument [6] was considered to be too specific.

In contrast to most other studies, this study allowed inclusion of patients with one previous episode of wheeze. Subgroup analyses of patients with no previous wheeze (n=264) showed similar results of the main outcome (LOS) as the total population (Table S3).

Table S1: Clinical score

	Score 0	**Score 1**	**Score 2**
Respiratory rate (breaths/min)	<40	40-60	>60
Respiratory chest recessions	None	Moderate Costodiaphragmatic	Severe As 1, +rib and jugular retraction
Auscultatory breath sounds	Vesicular	Wheeze + rales/ronchi	Faint ± severe wheeze ± pronounced rales and ronchi
Skin colour	Normal	Pallor	Cyanosis
General condition	Not affected	Moderately affected	Severely affected

Table S2: Randomization by hospital

Hospital	iRA	iS	OD	RI	Total
Oslo University Hospital	67	69	66	70	136
Østfold Hospital HF	52	48	49	51	100
Vestre Viken Hospital HF	15	13	14	14	28
Vestfold Hospital HF	17	19	17	19	38
Sykehuset Innlandet Lillehammer HF	11	12	14	9	23
Sykehuset Innlandet Elverum HF	15	13	14	14	28
Sørlandet Sykehus HF	10	12	12	10	22
Sykehuset Telemark HF	16	15	14	17	31

Table S3: Length of Stay in subgroup analysis

1 previous wheeze	**Adrenaline**	**Saline**	**On demand**	**Fixed schedule**
N	47	51	44	54
Mean LOS, hours	78.1	88.6	85.0	82.4
Estimated mean LOS*, hours (95% CI)	74.2	72.0	60.8	66.4
Mean difference in estimated mean LOS* (95% confidence interval)	-2.2 (-26.8,22.3) p=0.86		5.6 (-18.8,30.0) p=0.65	
No previous wheeze				
N	132	132	134	130
Mean LOS, hours	81.7	81.2	72.9	90.3
Estimated mean LOS*, hours (95% CI)	56.3	64.5	47.6	62.5
Mean difference in estimated mean LOS* (95% confidence interval)	8.3 (-4.8,21.3) p=0.22		14.9 (1.81,28.0) **p=0.03**	
Age <3 months				
N	92	85	86	91
Mean LOS, hours	100.9	91.4	83.5	108.5
Estimated mean LOS*, hours (95% CI)	93.1	89.2	41.3	71.6
Mean difference in estimated mean LOS* (95% confidence interval)	-3.8 (-24.7,17.0) p=0.72		30.3 (10.3,50.3) **p=0.003**	
Age > 3 months				
N	111	116	114	113
Mean LOS, hours	60.2	74.8	66.7	68.7
Estimated mean LOS*, hours (95% CI)	50.7	55.9	52.8	56.6
Mean difference in estimated mean LOS* (95% confidence interval)	5.2 (-6.7,17.0) p=0.39		3.8 (-8.0,15.6) p=0.53	

*Length of Stay (LOS) estimated by robust linear regression analyses.

Table S4: Results by all randomization groups

	Adrenaline/ On demand (N=102)	**Adrenaline / Fixed Schedule (N=101)**	**Saline/ On Demand (N=98)**	**Saline/ Fixed Schedule (N=103)**
Mean Length of Stay in hours (95% confidence intervals)	71.6 (58.9-84.2)	85.9 (72.0-99.7)	76.3 (62.9-89.8)	87.0 (74.5-99.6)
Change in clinical score by 1. Inhalations (95% confidence intervals)	-1.22 (-1.45 to -0.98)	-1.31 (-1.37 to 0.93)	-1.15 (-1.37 to -0.93)	-1.01 (-1.22 to -0.80)
Use for supportive therapy no/total no. (%)				
Oxygen	37/96 (38.5)	46/96 (47.9)	35/92 (38.0)	48/97 (49.5)
Nasogastric Tube Feeding	28/101 (27.7)	29/100 (29.0)	24/97 (24.7)	35/102 (34.3)
Ventilatory Support	2/102 (2.0)	13/101 (12.9)	6/98 (6.1)	9/103 (8.7)
Discontinued treatment	17/102 (16.7)	19/101 (18.8)	20/98 (20.4)	27/103 (26.2)

Table S5: Mean Length of Stay (LOS) for children not discontinued (n=321).

	Adrenaline/ On demand (N=85)	**Adrenaline/ Fixed Schedule (N=82)**	**Saline/ On Demand (N=78)**	**Saline/ Fixed Schedule (N=76)**
ITT: Mean LOS (hr)	57.5 (46.8-68.3)	69.4 (57.6-81.3)	59.5 (49.5-70.0)	76.4 (65.5-87.3)
PP: Mean LOS	54.7 (44.4-65.0)	64.0 (53.3-74.7)	54.8 (45.1-64.5)	72.8 (62.0-86.6)

	Adrenaline (N=167)	**Saline (N=154)**	**On Demand (N=163)**	**Fixed Schedule (N=158)**
Mean LOS actual discharge, hours (95% CI)	63.4 (55.3-71.4)	67.9 (60.3-75.4)	58.5 (51.1-65.9)	72.8 (64.7-80.9)
Mean LOS, deemed ready for discharge, hours (95% CI)	59.3 (51.8-66.7)	63.7 (56.3-71.0)	54.7 (47.6-61.8)	68.2 (60.6-75.8)
Estimated mean LOS*, actual discharge, hours (95% CI)	47.4 (31.4-63.4)	55.0 (39.9-70.1)	37.1 (21.1-53.0)	51.5 (35.3-67.7)
Mean difference in estimated mean LOS*, actual discharge	7.60 (-2.64 to 17.8), p=0.15		14.4 (4.3 to 24.6), **p=0.01**	
Estimated mean LOS*, deemed ready for discharge, hours (95% CI)	47.1 (31.6-62.6)	52.7 (45.4-58.9)	33.6 (18.2-49.1)	48.3 (39.3-57.3)
Mean difference in estimated mean LOS*, deemed ready for discharge	5.6 (-4.4 to 15.5), p=0.27		14.7 (4.9 to 24.5), **p=0.004**	
Mean number of inhalations (95% CI)	15.1 (13.0-17.2)	17.2 (14.8-19.6)	12.8 (10.9-14.6)	19.5 (17.0-22.0)

Pearsons correlation coefficient between actual time of discharge and deemed ready for discharge was calculated to 0.99. *Length of Stay (LOS) estimated by robust linear regression analyses.

Figure legends:

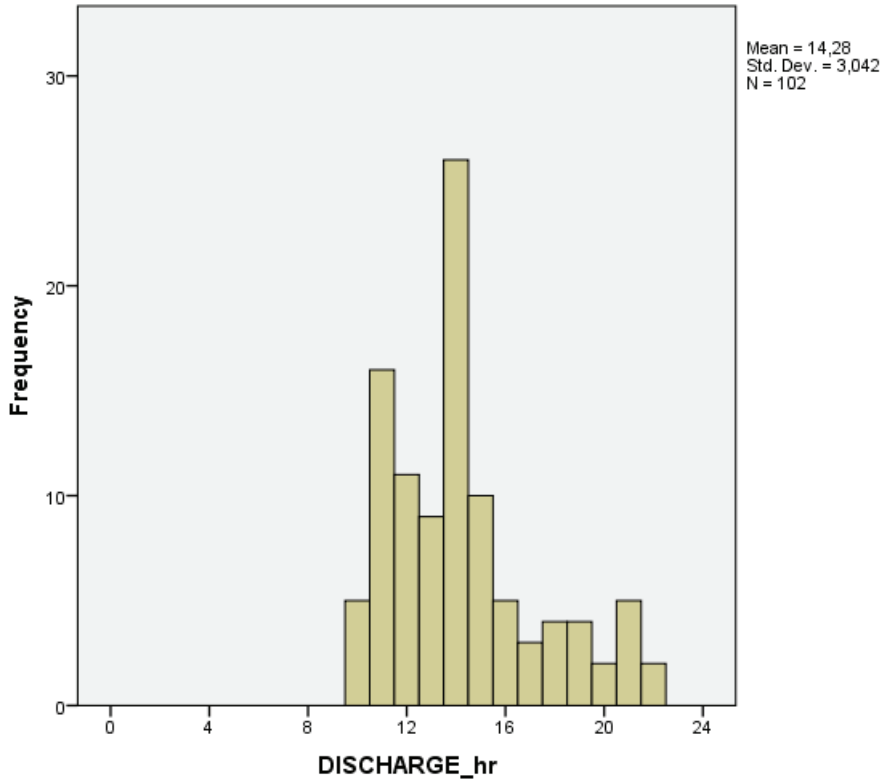
Figure S1: Discharge from hospital in number per hour of the day in children treated with bronchiolitis by randomization groups A (adrenaline/on demand), B (adrenaline/fixed schedule), C (saline/on demand) and D (saline/fixed schedule).

Figure S2: Length of Stay given by age for treatment medication with inhaled racemic adrenaline versus inhaled saline (A) and treatment strategy on demand versus fixed schedule (B) in infants with acute bronchiolitis. Regression lines are calculated by local regression smoothing, with 95% confidence interval shaded in gray for each of the smoothing lines.

References

1. Cunningham S, McMurray A. Observational study of two oxygen saturation targets for discharge in bronchiolitis. Archives of disease in childhood 2012;97:361-3.
2. Donlan M, Fontela PS, Puligandla PS. Use of continuous positive airway pressure (CPAP) in acute viral bronchiolitis: a systematic review. PediatrPulmonol 2011;46:736-46.
3. Wang EE, Law BJ, Stephens D. Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC) prospective study of risk factors and outcomes in patients hospitalized with respiratory syncytial viral lower respiratory tract infection. The Journal of pediatrics 1995;126:212-9.
4. Kristjansson S, Lodrup Carlsen KC, Wennergren G, Strannegard IL, Carlsen KH. Nebulised racemic adrenaline in the treatment of acute bronchiolitis in infants and toddlers. ArchDisChild 1993;69:650-4.
5. McCallum GB, Morris PS, Wilson CC, et al. Severity scoring systems: Are they internally valid, reliable and predictive of oxygen use in children with acute bronchiolitis? Pediatric pulmonology 2012.
6. Lowell DI, Lister G, Von Koss H, McCarthy P. Wheezing in infants: the response to epinephrine. Pediatrics 1987;79:939-45.

Figure S1a



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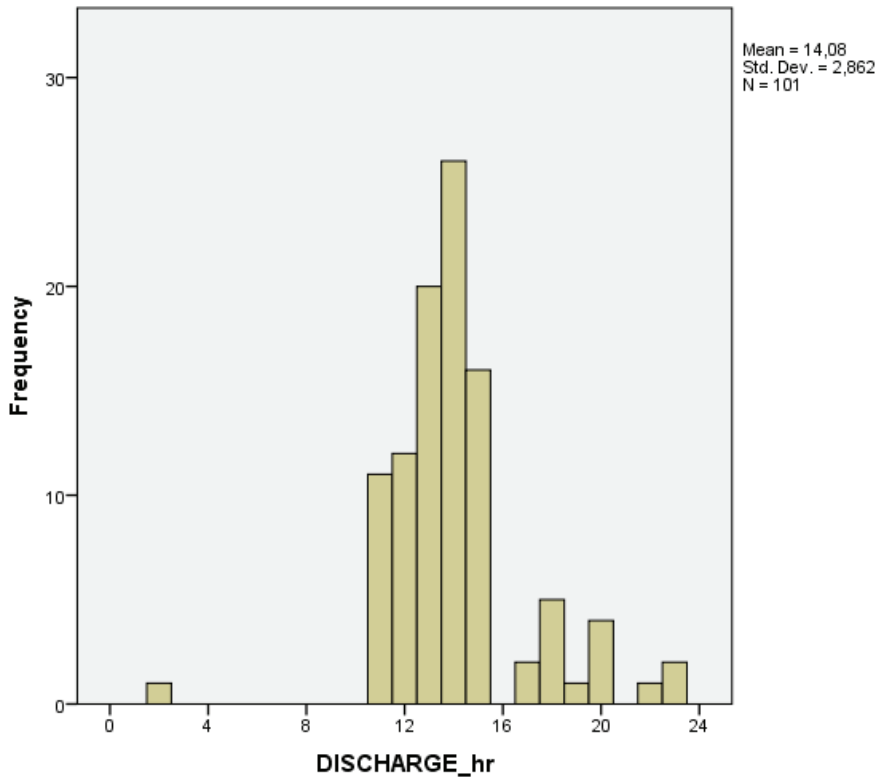

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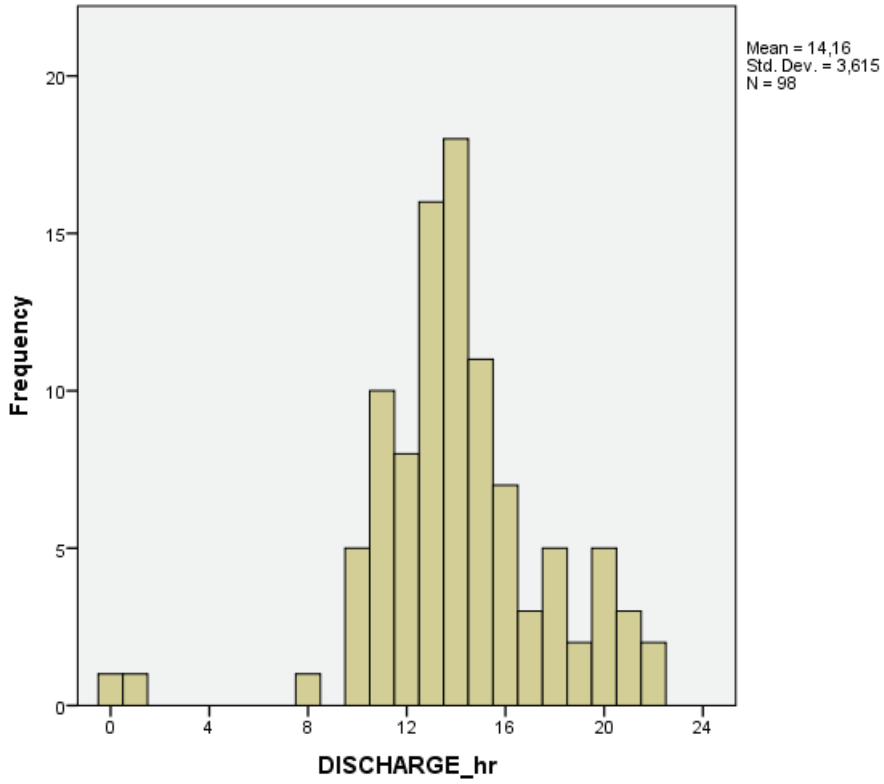

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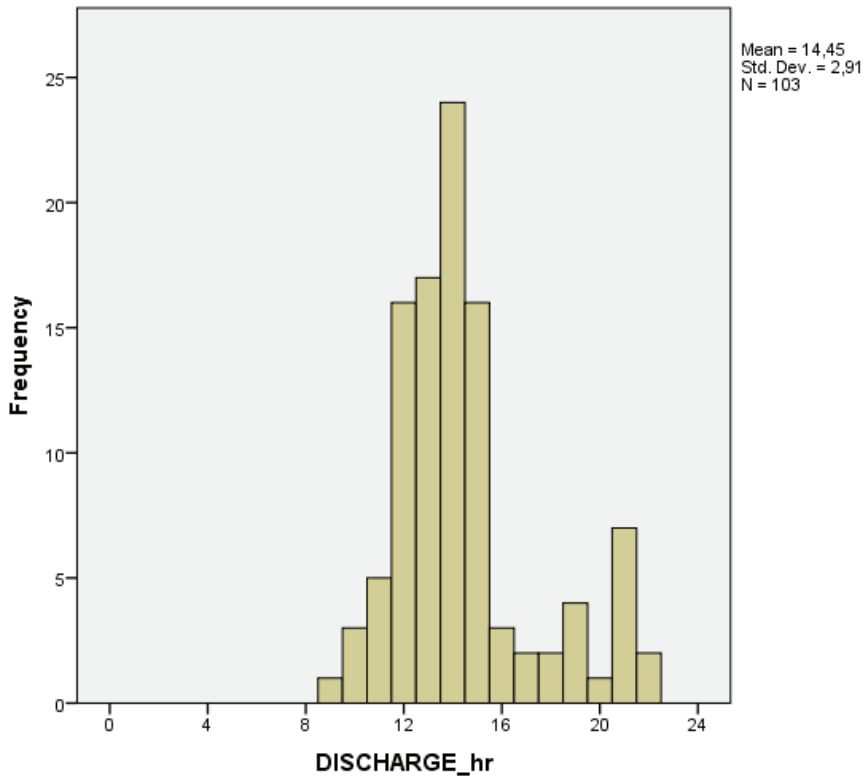
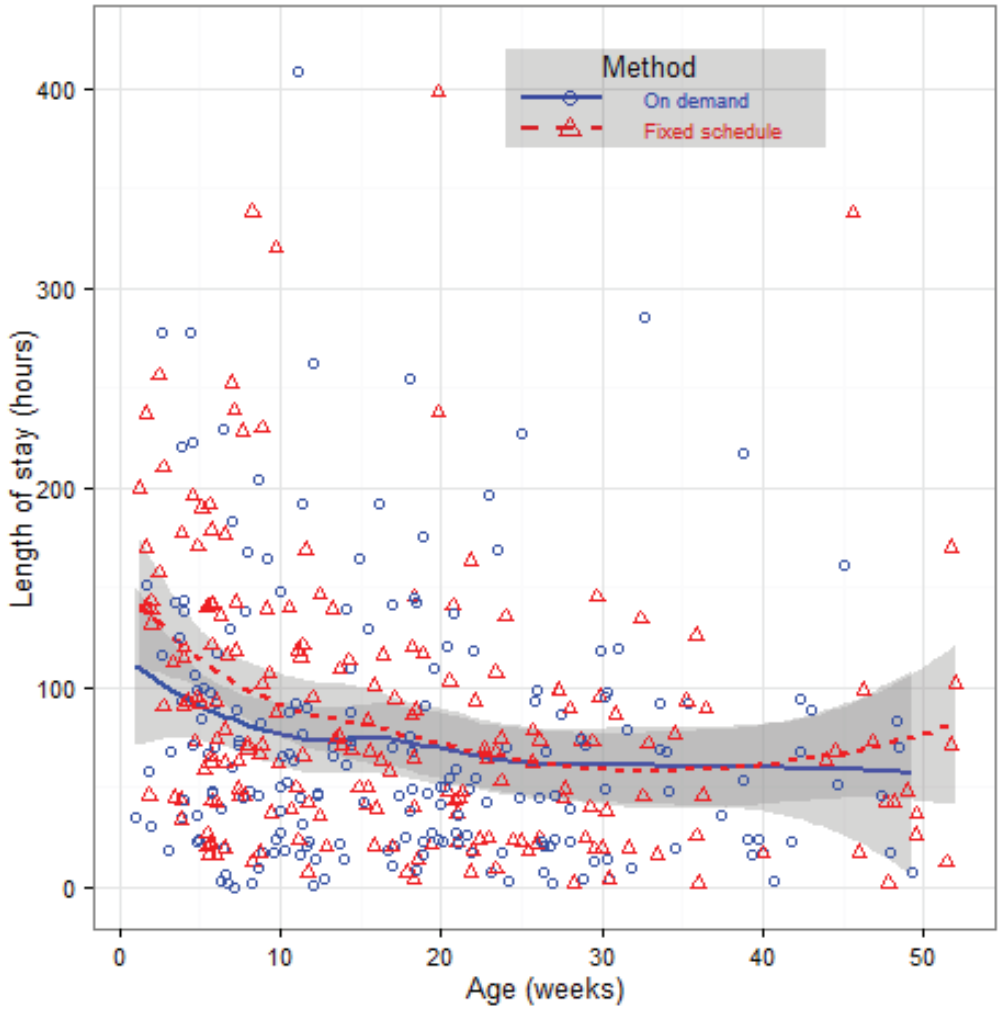


Figure S2a


Method
On demand
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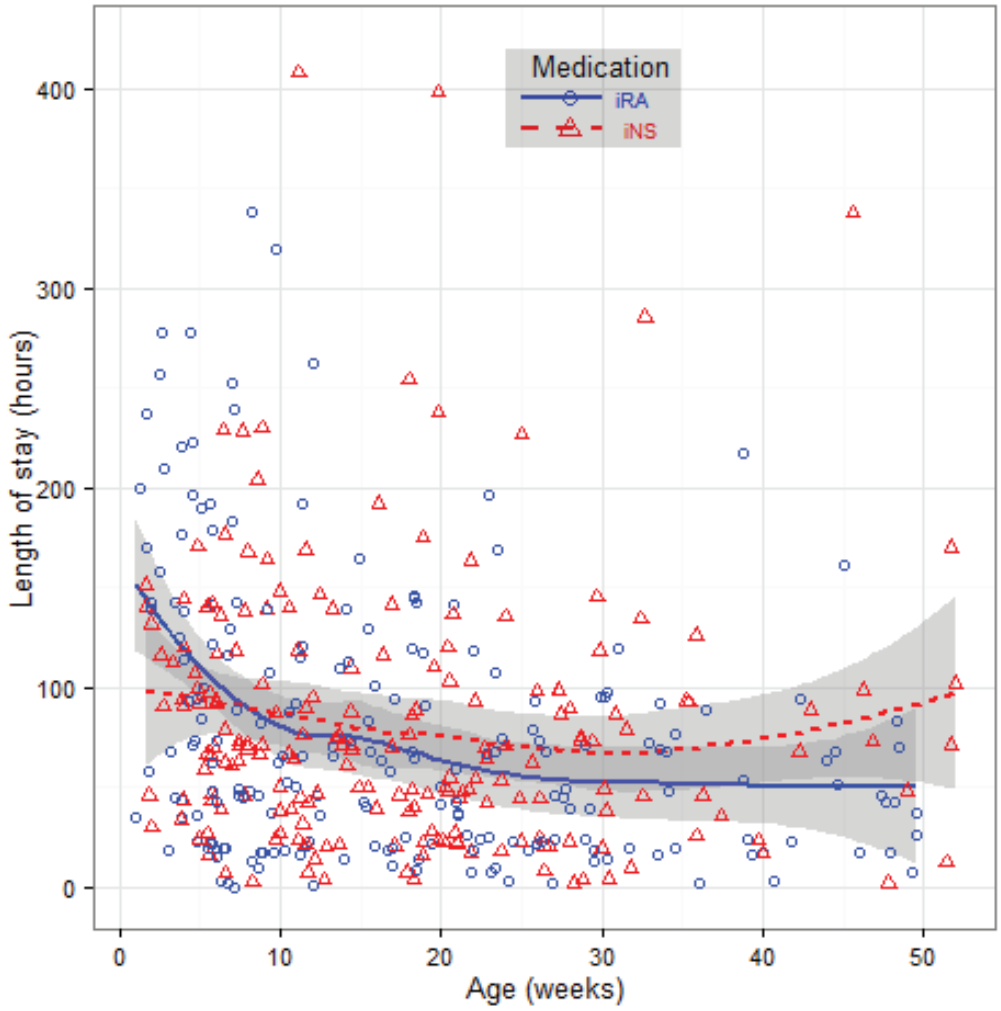
300
200
100
0
0
10
20
30
40
50
Age (weeks)

Figure S2b


Medication
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30
40
50
Age (weeks)

IV

ERJ open research

ORIGINAL ARTICLE
ASTHMA

Virus, allergic sensitisation and cortisol in infant bronchiolitis and risk of early asthma

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ABSTRACT

Background: Acute bronchiolitis during infancy and human rhinovirus (HRV) lower respiratory tract infections increases the risk of asthma in atopic children. We aimed to explore whether specific viruses, allergic sensitisation or cortisol levels during acute bronchiolitis in infancy increase the risk of early asthma, using recurrent wheeze as a proxy.

Methods: In 294 children with a mean (range) age of 4.2 (0–12) months enrolled during hospitalisation for acute infant bronchiolitis, we analysed virus in nasopharyngeal aspirates, serum specific immunoglobulin E against food and inhalant allergens, and salivary morning cortisol. These factors were assessed by regression analyses, adjusted for age, sex and parental atopy, for risk of recurrent wheeze, defined as a minimum of three parentally reported episodes of wheeze at the 2-year follow-up investigation.

Results: At 2 years, children with, compared to without, recurrent wheeze had similar rates of respiratory syncytial virus (RSV) (82.9% *versus* 81.8%) and HRV (34.9% *versus* 35.0%) at the acute bronchiolitis, respectively. During infancy, 6.9% of children with and 9.2% of children without recurrent wheeze at 2 years were sensitised to at least one allergen (p=0.5). Neither recurrent wheeze nor incidence rate ratios for the number of wheeze episodes at 2 years were significantly associated with specific viruses, high viral load of RSV or HRV, allergic sensitisation, or morning salivary cortisol level during acute bronchiolitis in infancy.

Conclusion: In children hospitalised with acute infant bronchiolitis, specific viruses, viral load, allergic sensitisation and salivary morning cortisol did not increase the risk of early asthma by 2 years of age.

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In infants with acute bronchiolitis, specific viruses including human rhinovirus, viral load and/or allergic sensitisation did not increase the risk of asthma by 2 years of age. http://bit.ly/2tCE9Yd

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Introduction

Acute bronchiolitis is the most common viral lower respiratory tract infection (LRTI) leading to hospitalisation in infants [1, 2], with respiratory syncytial virus (RSV) being the most commonly identified virus, followed by human rhinovirus (HRV) [3, 4]. Infants with moderate to severe bronchiolitis [5–9] and infants with atopic parents [7, 10] are reported to be at increased risk of developing recurrent wheeze and subsequent asthma. Both viral and host-specific factors may influence disease progression [6, 9]. A high genomic load of RSV has been associated with increasing disease severity [4, 11] but has not been well studied in relation to later wheeze and asthma.

Viral LRTI in children who are sensitised to allergens may increase the risk of childhood asthma development compared to LRTI in the absence of allergic sensitisation [10, 12]. A likely causal relationship between HRV in the presence of aeroallergen sensitisation and increased risk of wheeze, particularly at 1 year of age, has been suggested based upon a study among 285 high-risk children [13], while a Swedish cohort study reported that severe RSV bronchiolitis was a strong risk factor for allergic sensitisation later in childhood [14]. However, we are not aware of studies assessing the role of early allergic sensitisation in early infancy in risk of asthma, nor are we aware of allergic sensitisation being evaluated in terms for interactions with bronchiolitis for future asthma risk. We recently demonstrated that 8.4% of infants with acute bronchiolitis (4.2 months) were sensitised to at least on allergen [15].

The potential interaction between specific respiratory viruses and early allergic sensitisation in the development of asthma is therefore unclear, while studies assessing the role of allergic sensitisation and RSV/HRV infection in infants younger than 1 year for later asthma are largely lacking.

Increased levels of plasma and saliva cortisol during acute bronchiolitis [16, 17] may reflect the response to acute stress, while low cortisol levels are observed in children with asthma and allergic rhinitis [18, 19]. Stress, measured by serum cortisol levels, may therefore be involved in immune dysregulation, which may further increase airway inflammation through elevation of proinflammatory cytokines [20]. It is not clear if cortisol levels during acute bronchiolitis are associated with the subsequent development of asthma.

The aims of the present study were therefore to explore whether specific viruses, high viral load of RSV or HRV, allergic sensitisation, or morning salivary cortisol in infants with acute bronchiolitis increase the risk of early asthma.

Subjects and methods

Study design

The present study included all children enrolled in the multicentre, randomised clinical Bronchiolitis ALL South-East Norway trial during hospitalisation for acute bronchiolitis in infancy, and who attended a 2-year follow-up study [21]. Briefly, 404 infants with moderate to severe acute bronchiolitis <12 months of age were enrolled at the time of admission to one of eight hospitals in southeast Norway in 2010 and 2011 [22]. Inclusion criteria included clinical signs of bronchiolitis [23] and a clinical score [24] of at least 4 on a scale from 0 to 10, 10 indicating most severe disease (table S1). Infants with severe underlying disease, more than one previous episode of wheeze or 4 weeks persistent lower airway symptoms, or use of inhaled or systemic steroids in the last 4 weeks were excluded.

At enrolment, we performed a structured parental interview, and collected nasopharyngeal aspirates for viral analyses, blood samples for specific immunoglobulin E (s-IgE) analyses and morning salivary sample for cortisol analyses. The 2-year follow-up visit included parental structured interviews for detailed medical history and a clinical investigation.

Caregivers of all infants provided informed written consent at enrolment. The study was approved by the Regional Committees for Medical and Health Research Ethics and by the Norwegian Medicine Agency. The Bronchiolitis All South-East Norway study is registered at www.clinicaltrials.gov (NCT00817466) and EudraCT (2009-012667-34), and is registered in the Norwegian Biobank Registry. The study was audited by the Norwegian Medicines Agency in 2011.

Subjects

The baseline characteristics of the 294 children who attended the 2-year follow-up visit were not significantly different from the 110 nonattendees, apart from a higher maternal ($p<0.001$) and paternal ($p=0.019$) education (table S2).

Methods

Structured interview by study physicians with caregivers at both visits addressed the previous and current health of the child and family members, parental atopy, socioeconomic factors and ethnicity, with a focus on episodes of bronchial obstruction (wheeze). Parental education was categorised into five levels; from 1

(no school completed) to 5 (higher education, >3 years), with further details provided in the supplementary material.

Wheezing episodes were reported based upon the questions, "Has the child had episodes of wheezing and/or chest tightness? If yes: specify the total number of episodes from birth to enrolment (first assessment) and from enrolment to the two-year assessment." Additionally, parents were asked if the child had doctor-diagnosed asthma.

Virus analyses including RSV and HRV, as well as HRV subtypes A/B and C, were performed using real-time PCR at the Dept of Allergy, University of Athens (Athens, Greece), as described in detail elsewhere [4]. As previously described in detail, high viral genomic load was identified using data-driven cluster analyses restricted to five clusters and classified as high *versus* all other concentration clusters per virus [4].

Blood samples were collected by venepuncture and analyses of s-IgE were performed at Fürst Medical Laboratory (Oslo, Norway) using ImmunoCAP (Phadia AB, Uppsala, Sweden). We first screened for allergic sensitisation with Phadiatop Infant, and in all samples proceeding with further analyses in all samples with serum s-IgE $\geqslant$0.15 kU·L^{-1} (n=89), for s-IgE to hen's egg white, cow's milk, peanut, cat, dog, birch, timothy and house dust mite. All analyses were performed in one batch and specified down to 0.10 kU·L^{-1}. Allergic sensitisation was defined as s-IgE $\geqslant$0.35 kU·L^{-1} to at least one allergen. Results from analyses using the cut-off level of 0.1 kU·L^{-1} are provided in the table S3 [15].

Salivary cortisol sampling was performed the first morning after hospital admission, as soon as possible after the child's awakening (after 06:00 h) and before the child's first meal, using two sorbettes (hydrocellulose; Salimetrics Europe Ltd, Suffolk, UK) as previously described in detail [25]. The sorbettes were then frozen at −86°C until transferred to Karolinska Institutet (Stockholm, Sweden) for analysis by radioimmunoassay [17].

Outcomes and potential explanatory variables

The primary outcome was early asthma by use of the proxy recurrent wheeze at 2 years, defined as at least three episodes of wheeze, including the acute bronchiolitis at enrolment. We also used the total number of wheezing episodes by 2 years of age.

The main explanatory variables were: the presence of RSV, HRV, and HRV subtypes A/B and C in nasopharyngeal secretions; viral genomic load of RSV and HRV; allergic sensitisation to any allergen, any food allergen or any inhalant allergen, or to single allergens; as well as salivary morning cortisol level. Sex, age, parental atopy and time from birth to the time of the 2-year follow- up, hereafter referred to as exposure time, were used as covariates.

Statistical analyses

Continuous data were analysed using Students t-test and are presented as mean values with standard deviations, ranges or 95% confidence intervals, where appropriate. Categorical data, analysed by Pearson Chi-squared test, are given as numbers and percentages.

The analyses focused on the total number of wheezing episodes experienced by 2 years of age, including the acute bronchiolitis at enrolment. A dichotomisation with at least three events was used as a proxy for early asthma and served as our primary outcome. It was analysed by a logistic regression.

A secondary analysis focused on the actual counts of wheezing episodes. According to the inclusion criterion, all children had at least one incidence and therefore a zero-truncated Poisson regression would be a natural candidate for this analysis. Due to overdispersion, the zero-truncated negative binomial model was, however, found more suitable. Both models used the same set of explanatory variables: the presence of RSV, HRV, and HRV subtypes A, B and C; viral genomic load of RSV and HRV; allergic sensitisation to any allergen, any food allergen or any inhalant allergen, or to single allergens as well as salivary morning cortisol level. Relevant interaction analyses were performed. Both models were adjusted for age, sex and parental atopy. The exposure time, given by the time from birth to the time of the 2-year follow-up, was used as an offset.

The significance level was set to 0.05 (5%). Analyses were performed using IBM SPSS version 25.0 (IBM, Armonk, NY, USA.) and R 3.6.0 (The R Foundation, Vienna, Austria).

Results

Baseline and clinical characteristics at enrolment of the 294 children are shown in table 1. The mean age (range) was 4.2 (0–12) months at enrolment and 24.5 (9.7–35.2) months at the 2-year follow-up visit.

The most commonly identified virus was RSV, in 82% of cases, with a high genomic load observed in 54%; followed by HRV, identified in 35% (table 1 and figure S1).

Allergic sensitisation was present in 8.1% of the infants, with food allergen sensitisation in 7.4% and 1.8% being sensitised to inhalant allergens. 13 infants (4.8%) were monosensitised (table 1).

Salivary cortisol was available for 133 of the infants, with a geometric mean (95% CI) of 42.0 (32.9–53.7) mmol·L^{-1}.

TABLE 1 Characteristics at birth, study enrolment and 2 years of age are reported stratified by the presence or absence of recurrent wheeze at 2 years of age

	Recurrent wheeze	No recurrent wheeze	p-value
Participants	143 (48.6%)	151 (51.4%)	
At birth			
Male sex	96/143 (67.1%)	85/151 (56.3%)	0.06
GA weeks	38.6±2.4	38.5±4.0	0.95
Born at GA <37 weeks	14/110 (12.7%)	15/116 (12.9%)	0.96
Birth weight g	3347±632	3497±642	0.05
At enrolment			
Age days mean (range)	134 (14–348)	115 (7–363)	0.05
Weight g	6650±1886	6245±1794	0.06
Eczema	15/131 (11.5%)	14/140 (10.0%)	0.70
One previous episode of wheeze	34/127 (26.8%)	35/133 (26.3%)	0.93
Length of hospital stay h	85±72	76±59	0.16
Need of supportive treatment	73/142 (51.4%)	79/150 (52.7%)	0.83
Virus detected during acute bronchiolitis			
RSV	107/129 (82.9%)	112/137 (81.8%)	0.80
HRV	45/129 (34.9%)	48/137 (35.0%)	0.98
HRV A or B	12/129 (9.3%)	16/137 (11.7%)	0.53
HRV C	33/129 (25.6%)	32/137 (23.4%)	0.67
RSV high genomic load	68/129 (52.7%)	77/137 (56.2%)	0.57
HRV high genomic load	8/129 (6.2%)	8/137 (5.8%)	0.90
More than one virus	80/129 (62.0%)	90/137 (65.7%)	0.53
Allergic sensitisation[#]			
Any sensitisation	9/130 (6.9%)	13/141 (9.2%)	0.49
Any food sensitisation	8/130 (6.2%)	12/141 (8.5%)	0.46
Any inhalant sensitisation	3/130 (2.3%)	2/141 (1.4%)	0.59
Egg sensitisation	5/130 (3.8%)	3/141 (2.1%)	0.40
Cow's milk sensitisation	4/130 (3.1%)	9/141 (6.4%)	0.20
Peanut sensitisation	1/141 (0.7%)	2/130 (1.5%)	0.52
Polysensitisation	4/130 (3.1%)	3/141 (2.1%)	0.62
Monosensitisation	4/130 (3.1%)	9/141 (6.4%)	0.20
Cortisol mmol·L^{-1} geometric mean (95% CI)	37.0 (30.2–45.4)	33.7 (28.5–39.8)	
At the 2-year follow-up			
Age days mean (range)	747 (291–1055)	725 (368–979)	0.07
Asthma diagnosed by physician	51/143 (35.7%)	5/151 (3.3%)	<0.001
Asthma medication used	108/134 (80.6%)	28/142 (19.7%)	<0.001
Parental education			
Maternal education[¶]	3.93±0.94	4.05±1.03	0.31
Paternal education[¶]	3.76±0.97	3.92±1.02	0.21
Parental allergic diseases			
Any[+]	70/128 (54.7%)	60/136 (44.1%)	0.09
Maternal asthma	22/112 (19.6%)	14/123 (11.4%)	0.08
Paternal asthma	16/112 (14.3%)	15/123 (12.2%)	0.64
Maternal rhinoconjunctivitis	21/124 (16.9%)	21/134 (15.7%)	0.78
Paternal rhinoconjunctivitis	31/124 (25.0%)	25/134 (18.7%)	0.22
Maternal eczema	20/128 (15.6%)	11/135 (8.1%)	0.06
Paternal eczema	15/128 (11.7%)	10/135 (7.4%)	0.23
Environment			
Smoking at home	19/124 (15.3%)	19/129 (14.7%)	0.90

Data are presented as n/N (%) or mean±SD, unless otherwise stated. GA: gestational age; RSV: respiratory syncytial virus; HRV: human rhinovirus. [#]: specific immunoglobulin E ⩾0.35 kU·L^{-1}; [¶]: categorised from 1 (no school completed) to 5 (higher education, >3 years); [+]: reported asthma, eczema and/or rhinoconjunctivitis.

At the 2-year follow-up investigation, 49% had recurrent wheeze, while doctor-diagnosed asthma was reported for 56 (19%) children. The mean±SD number of wheeze episodes was 5.25±7.4 at 2 years of age and 49% had used at least one asthma medication during the last year (table 1).

Recurrent wheeze by 2 years of age was neither significantly associated with RSV or HRV, nor with rates of high viral load of RSV or HRV during acute infant bronchiolitis (figure 1a and table 1). The rate of HRV C at the time of acute bronchiolitis was similar in children with recurrent wheeze (25.6%) and without recurrent wheeze (23.4%) (p=0.7).

Allergic sensitisation at enrolment was not significantly different among infants with (7.4%) compared to those without recurrent wheeze (9.2%) (p=0.49) (figure 1b). Similar results were observed using analyses with cut-off values of allergic sensitisation of 0.10 $kU \cdot L^{-1}$ (table S3).

Morning salivary cortisol levels in infancy were similar among children who, at 2 years of age, had recurrent wheeze and children who did not (geometric mean (95% CI) 33.7 (28.5–39.8) *versus* 37.0 (30.2–45.4) $nmol \cdot L^{-1}$, respectively) (figure 1c).

In multivariate analyses adjusted for age, sex, parental atopy and time since enrolment, recurrent wheeze at 2 years of age was not significantly associated with specific viruses, high viral load of RSV or HRV, allergic sensitisation, or morning salivary cortisol level in infancy (table 2). We found no significant interactions between specific viruses, viral load sensitisation and salivary cortisol levels.

The zero-truncated negative binomial regression analysis showed no significant effects upon the incidence rate ratios for recurrent wheeze of any specific viruses, high viral load of RSV or HRV, allergic sensitisation, or morning salivary cortisol level (table 3). No significant interactions between specific viruses, viral load sensitisation and salivary cortisol levels were further identified.

Discussion

Recurrent wheeze at 2 years of age was not significantly associated with specific viruses, high viral load of RSV or HRV, allergic sensitisation, or morning salivary cortisol during acute bronchiolitis leading to hospitalisation in the first year of life.

The lack of associations between recurrent wheeze at 2 years of age and type of virus identified during the acute bronchiolitis in infancy in our study is supported by a Swedish and Norwegian study reporting that the risk of asthma and recurrent wheeze at follow-up in early childhood was independent of the presence of RSV during acute bronchiolitis [9, 26]. Furthermore, recurrent wheeze at 5 years of age was similar among HRV and RSV associated wheeze during infancy in 198 high-risk infants in an Australian birth cohort [10]. In contrast, a recent review [5] and others [7, 27] suggests that HRV-induced LRTI increase future asthma risk compared to other viruses, in line with a suggestion that the epithelial barrier function in the airways may be impaired with HRV infection, initiating an airway inflammatory response [28]. The apparent differences in associations between HRV *versus* RSV and later wheeze in our, compared to an Italian [29] and a Finnish study [30], may be related to differences in the detected viruses at the acute bronchiolitis. While we found HRV in 6%, RSV in 53% and both in 29% (figure S1), the Italian study of 230 infants using nasal washes identified HRV in 9%, RSV in 43% and both in 0.4%, and the Finnish study of 329 infants found the corresponding rates of 11%, 63% and 3% in nasopharyngeal aspirates, respectively. Thus, the analytic sensitivity of viral detection or the viral profile among the populations may influence the likelihood of detecting risk differences for later recurrent wheeze. Furthermore, the young age of our study population compared to infants older than 1 year may reflect different pathophysiological mechanisms by which LRTI increases the risk of future asthma. The bronchiolitis cohort studied by SIGURS *et al.* [14] included infants with RSV bronchiolitis only and healthy controls. This may have contributed to a misconception that increased risk of asthma development was specific for RSV bronchiolitis.

The lack of association between HRV C and recurrent wheeze at 2 years of age in the present study is in contrast to the increased risk of later hospitalisation for respiratory illness in HRV C positive infants [31], and increased severity of acute asthma in children with HRV C infections compared to HRV A and B [32].

The finding in the present study that high RSV and HRV genomic loads were not significantly associated with subsequent recurrent wheeze at 2 years of age is, to the best of our knowledge, novel. A high viral load appears to cause more severe bronchiolitis, as shown in the Bronchiolitis All study and by others [4, 11], but we are not aware of studies investigating the association between viral load during acute infection and later recurrent wheeze and asthma.

Our finding that allergic sensitisation in early infancy was not associated with early asthma does not support the suggestion that early recognition of s-IgE sensitisation against common food and aeroallergens may identify wheezing children who are at risk of asthma development [33]. To the best of our

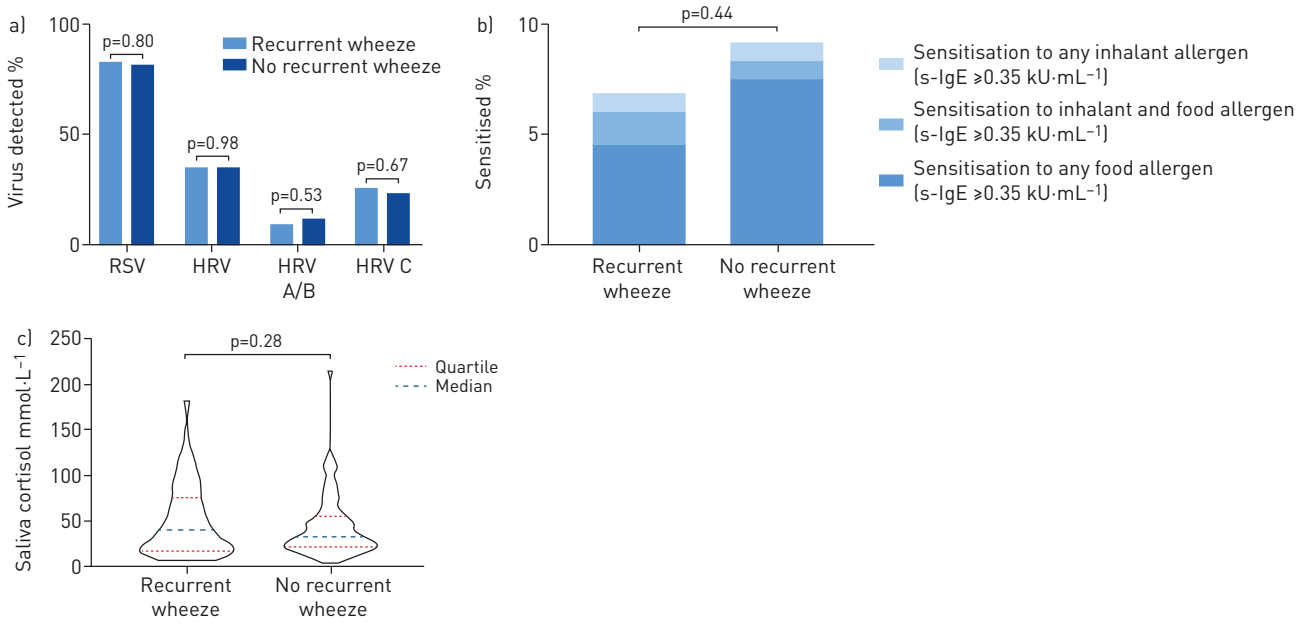

FIGURE 1 a) The distribution of respiratory syncytial virus (RSV), human rhinovirus (HRV), and HRV species A and B, and C; b) the distribution of sensitisation to inhalant and food allergens; and c) the distribution of morning saliva cortisol sampled the first morning after hospitalisation, in infants hospitalised with acute bronchiolitis at study enrolment (0–12 months of age, mean age 4.2 months), compared to children with recurrent wheeze and no recurrent wheeze at 2-year follow-up. s-IgE: specific immunoglobulin E.

knowledge, this is the first study to explore whether allergic sensitisation at the time of acute infant bronchiolitis increases the risk of recurrent wheeze. Our results are supported by a community-based study of 263 high-risk children that failed to show that a positive skin-prick test at 6 months of age increased the risk of recurrent wheeze at 2 years of age, while persistent wheeze at 5 years was associated with sensitisation first at 2 years of age [10]. Conversely, in a Finnish randomised controlled trial enrolling 3–23-month-old children with their first episode of wheeze, allergic sensitisation at a mean age of

TABLE 2 The odds ratios of having recurrent wheeze at 2 years of age are shown by factors observed among infants (0–12 months of age) who were admitted to hospital with acute bronchiolitis

	Recurrent wheeze OR (95% CI)	p-value
Viral detection during acute bronchiolitis		
RSV	0.91 (0.45–1.83)	0.78
HRV	1.03 (0.59–1.78)	0.93
HRV A or B	0.74 (0.31–1.8)	0.51
HRV C	1.19 (0.65–2.19)	0.58
RSV high genomic load	0.84 (0.50,1.42)	0.52
HRV high genomic load	0.93 (0.30–2.84)	0.90
Multiple viruses	0.83 (0.47–1.44)	0.50
Allergic sensitisation#		
Any sensitisation	0.72 (0.28–1.89)	0.51
Any food sensitisation	0.71 (0.25–1.96)	0.50
Any inhalant sensitisation	1.08 (0.19–20.89)	0.56
Egg sensitisation	1.33 (0.27–6.45)	0.72
Cow's milk sensitisation	0.41 (0.10–1.64)	0.21
Peanut sensitisation	0	0.99
Polysensitisation	1.61 (0.29–32.79)	0.35
Salivary morning cortisol	1.00 (0.99–1.01)	0.40

Bivariate odds ratios are adjusted for sex, age at inclusion and parental atopy. RSV: respiratory syncytial virus; HRV: human rhinovirus. #: specific immunoglobulin E ⩾0.35 kU·L^{-1}.

TABLE 3 The incidence rate ratio (IRR) for episodes of recurrent wheeze at 2 years of age are given by factors observed in infants admitted to hospital with acute bronchiolitis, based on zero-truncated negative binomial regression analysis

	Recurrent wheeze IRR (95% CI)	p-value
Viral detection during acute bronchiolitis		
RSV	0.95 (0.70–1.28)	0.86
HRV	0.72 (0.57–0.91)	0.16
HRV A or B	0.58 (0.40–0.83)	0.14
HRV C	0.84 (0.65–1.09)	0.51
RSV high genomic load	0.92 (0.73–1.15)	0.70
HRV high genomic load	0.61 (0.38–0.99)	0.31
Multiple viruses	0.91 (0.72–1.16)	0.69
Allergic sensitisation#		
Any sensitisation	1.46 (0.97–2.19)	0.36
Any food sensitisation	1.53 (1.0–2.36)	0.32
Any inhalant sensitisation	1.18 (0.48–2.90)	0.85
Egg sensitisation	3.58 (1.82–3.58)	0.059
Cow's milk sensitisation	0.49 (0.29–0.95)	0.17
Peanut sensitisation	1.45 (0.25–8.33)	0.83
Polysensitisation	1.44 (0.60–3.49)	0.68
Salivary morning cortisol	1.01 (1.0–1.0)	0.56

The zero-truncated negative binomial regression analysis are adjusted for sex, age at inclusion and parental atopy. RSV: respiratory syncytial virus; HRV: human rhinovirus.#: specific immunoglobulin E $\geqslant$0.35 kU·L^{-1}.

11 months was associated with increased risk of asthma at 8 years of age [34]. Like our study, most children in the Finnish study were admitted to hospital. However, the rate of allergic sensitisation in our study was 8% with only 2% being sensitised to inhalant allergens, while 17% in the Finnish study were sensitised to any allergen and 5% to inhalant allergens. As previously shown in this cohort [15], infants older than, compared to younger than, 4 months were significantly more often sensitised to allergens. Thus, the young age of the infants in the present study probably represents a time at which most infants have not started to develop s-IgE to relevant allergens increasing the risk of asthma. In the COAST (Childhood Origins of Asthma) birth cohort study [13], high-risk children sensitised to aeroallergens were at increased risk of wheezing illness caused by HRV, but s-IgE was not analysed before 1 year of age.

No significant interaction was observed between HRV bronchiolitis and allergic sensitisation for recurrent wheeze in the present study. Our novel finding contrasts with the increased risk of asthma observed among children with HRV wheezy illness who were sensitised to allergens, compared to those that were not [10, 13]. The contradictory result may suggest that the underlying mechanisms in acute bronchiolitis may differ from those in older children or may be related to a difference in hereditary risk in the study populations [7, 10, 13]. Our study does, therefore, not support the hypothesis that recurrent wheeze, which is triggered by HRV infection in susceptible infants, is conferred by early allergic sensitisation [10, 12].

Apart from ours, few studies have assessed the risk of recurrent wheeze related to potential subgroups of bronchiolitis; younger infants with predominantly RSV, a phenotype of HRV in atopic infants and bronchiolitis due to other virus [35]. Clearly, more studies are needed to provide further insight into the role of early allergic sensitisation and viral LRTI in asthma development.

We found no association between cortisol level in infants hospitalised with acute bronchiolitis and recurrent wheeze at 2 years of age, in contrast to the lower saliva cortisol level observed in children with asthma [18]. Previous results in this cohort showed that infants with acute bronchiolitis had higher levels of morning salivary cortisol than controls at enrolment, but similar cortisol levels at 2 years of age [17].

The present study is strengthened by the prospective design with a 2-year follow up study in a cohort of well-characterised infants hospitalised with acute bronchiolitis. The rate of recurrent wheeze of 47% is in line with others [7, 10, 36, 37]. The study was based on physician-led interviews instead of self-reported questionnaires. Parentally reported wheezing episodes may not, in all cases, have been documented by a physician, which is a potential limitation in this study. However, >80% of the children with, *versus* 20% without, recurrent wheeze reported to have used asthma medication, supporting the use of recurrent wheeze as a proxy for early asthma. A Swedish study, initially reported by Wennergren *et al.* [9], reported increased risk of asthma maintained up to 27 years of age [38]

Although the size of the study population is reasonable in relation to similar studies, the low number of children with allergic sensitisations in this age group limits the ability of the present study to detect significant interactions with HRV or RSV. A longer follow-up period could have provided further insight into the role of early sensitisation in early viral infection and asthma development.

In conclusion, in children hospitalised with acute bronchiolitis during the first year of life, specific viruses and viral load, allergic sensitisation, and salivary morning cortisol did not increase the risk of early asthma. It remains unclear to what degree allergic sensitisation in early infancy may contribute to early asthma development after viral LRTI.

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References

1 Wright AL, Taussig LM, Ray CG, *et al.* The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. *Am J Epidemiol* 1989; 129: 1232–1246.
2 Meissner HC. Viral bronchiolitis in children. *N Engl J Med* 2016; 374: 1793–1794.
3 Mansbach JM, Piedra PA, Teach SJ, *et al.* Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. *Arch Pediatr Adolesc Med* 2012; 166: 700–706.
4 Skjerven HO, Megremis S, Papadopoulos NG, *et al.* Virus type and genomic load in acute bronchiolitis: severity and treatment response with inhaled adrenaline. *J Infect Dis* 2016; 213: 915–921.
5 Jartti T, Gern JE. Role of viral infections in the development and exacerbation of asthma in children. *J Allergy Clin Immunol* 2017; 140: 895–906.
6 Martinez FD, Morgan WJ, Wright AL, *et al.* Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. *N Engl J Med* 1988; 319: 1112–1117.
7 Lemanske RF Jr. Jackson DJ, Gangnon RE, *et al.* Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005; 116: 571–577.
8 Sigurs N, Aljassim F, Kjellman B, *et al.* Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax* 2010; 65: 1045–1052.
9 Wennergren G, Hansson S, Engstrom I, *et al.* Characteristics and prognosis of hospital-treated obstructive bronchitis in children aged less than two years. *Acta Paediatr* 1992; 81: 40–45.
10 Kusel MM, de Klerk NH, Kebadze T, *et al.* Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007; 119: 1105–1110.
11 DeVincenzo JP, El Saleeby CM, Bush AJ. Respiratory syncytial virus load predicts disease severity in previously healthy infants. *J Infect Dis* 2005; 191: 1861–1868.
12 Holt PG, Upham JW, Sly PD. Contemporaneous maturation of immunologic and respiratory functions during early childhood: implications for development of asthma prevention strategies. *J Allergy Clin Immunol* 2005; 116: 16–24.
13 Jackson DJ, Evans MD, Gangnon RE, *et al.* Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med* 2012; 185: 281–285.
14 Sigurs N, Bjarnason R, Sigurbergsson F, *et al.* Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000; 161: 1501–1507.
15 Skjerven HO, Gjengsto Hunderi JO, Carlsen KH, *et al.* Allergic sensitisation in infants younger than one year of age. *Pediatr Allergy Immunol* 2019; 31: 103–206.
16 Pinto RA, Arredondo SM, Bono MR, *et al.* T helper 1/T helper 2 cytokine imbalance in respiratory syncytial virus infection is associated with increased endogenous plasma cortisol. *Pediatrics* 2006; 117: e878–e886.
17 Rolfsjord LB, Bakkeheim E, Berents TL, *et al.* Morning salivary cortisol in young children: reference values and the effects of age, sex, and acute bronchiolitis. *J Pediatr* 2017; 184: 193–198.
18 Bakkeheim E, Mowinckel P, Carlsen KH, *et al.* Reduced basal salivary cortisol in children with asthma and allergic rhinitis. *Acta Paediatr* 2010; 99: 1705–1711.
19 Landstra AM, Postma DS, Boezen HM, *et al.* Role of serum cortisol levels in children with asthma. *Am J Respir Crit Care Med* 2002; 165: 708–712.
20 Priftis KN, Papadimitriou A, Nicolaidou P, *et al.* Dysregulation of the stress response in asthmatic children. *Allergy* 2009; 64: 18–31.
21 Skjerven HO, Rolfsjord LB, Berents TL, *et al.* Allergic diseases and the effect of inhaled epinephrine in children with acute bronchiolitis: follow-up from the randomised, controlled, double-blind, Bronchiolitis ALL trial. *Lancet Respir Med* 2015; 3: 702–708.

22 Skjerven HO, Hunderi JO, Brugmann-Pieper SK, *et al.* Racemic adrenaline and inhalation strategies in acute bronchiolitis. *N Engl J Med* 2013; 368: 2286–2293.
23 Court SD. The definition of acute respiratory illnesses in children. *Postgrad Med J* 1973; 49: 771–776.
24 Kristjansson S, Lodrup Carlsen KC, Wennergren G, *et al.* Nebulised racemic adrenaline in the treatment of acute bronchiolitis in infants and toddlers. *Arch Dis Child* 1993; 69: 650–654.
25 Tryphonopoulos PD, Letourneau N, Azar R. Approaches to salivary cortisol collection and analysis in infants. *Biol Res Nurs* 2014; 16: 398–408.
26 Fjaerli HO, Farstad T, Rod G, *et al.* Acute bronchiolitis in infancy as risk factor for wheezing and reduced pulmonary function by seven years in Akershus County, Norway. *BMC Pediatr* 2005; 5: 31.
27 Lehtinen P, Ruohola A, Vanto T, *et al.* Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. *J Allergy Clin Immunol* 2007; 119: 570–575.
28 Looi K, Troy NM, Garratt LW, *et al.* Effect of human rhinovirus infection on airway epithelium tight junction protein disassembly and transepithelial permeability. *Exp Lung Res* 2016; 42: 380–395.
29 Midulla F, Nicolai A, Ferrara M, *et al.* Recurrent wheezing 36 months after bronchiolitis is associated with rhinovirus infections and blood eosinophilia. *Acta Paediatr* 2014; 103: 1094–1099.
30 Bergroth E, Aakula M, Korppi M, *et al.* Post-bronchiolitis use of asthma medication: a prospective 1-year follow-up study. *Pediatr Infect Dis J* 2016; 35: 363–368.
31 Cox DW, Bizzintino J, Ferrari G, *et al.* Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. *Am J Respir Crit Care Med* 2013; 188: 1358–1364.
32 Bizzintino J, Lee WM, Laing IA, *et al.* Association between human rhinovirus C and severity of acute asthma in children. *Eur Respir J* 2011; 37: 1037–1042.
33 Sly PD, Boner AL, Bjorksten B, *et al.* Early identification of atopy in the prediction of persistent asthma in children. *Lancet* 2008; 372: 1100–1106.
34 Lukkarinen M, Koistinen A, Turunen R, *et al.* Rhinovirus-induced first wheezing episode predicts atopic but not nonatopic asthma at school age. *J Allergy Clin Immunol* 2017; 140: 988–995.
35 Jartti T, Smits HH, Bonnelykke K, *et al.* Bronchiolitis needs a revisit: distinguishing between virus entities and their treatments. *Allergy* 2019; 74: 40–52.
36 Sigurs N, Bjarnason R, Sigurbergsson F, *et al.* Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. *Pediatrics* 1995; 95: 500–505.
37 Stein RT, Sherrill D, Morgan WJ, *et al.* Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999; 354: 541–545.
38 Goksor E, Amark M, Alm B, *et al.* High risk of adult asthma following severe wheezing in early life. *Pediatr Pulmonol* 2015; 50: 789–797.

Supplementary material to the manuscript:

Virus, allergic sensitisation and cortisol in infant bronchiolitis and risk of early asthma

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A study performed within ORAACLE (the Oslo Research Group of Asthma and Allergy in Childhood; the Lung and Environment).

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SUBJECTS AND METHODS

Methods

Educational level was categorized into 1- no school completed, 2- primary school completed, 3- secondary school completed, 4- higher education, up to three years and 5 higher education, more than three years.

Nasopharyngeal aspirates were collected using standardized procedure performed by trained paediatric nurses at study inclusion, using a tracheal suction set, and immediately frozen at -20°C.

Table S1 The Clinical Score used in the study (1), Kristjansson et.al**.**

	Score 0	**Score 1**	**Score 2**
Respiratory rate (breaths/min)	<40	40-60	>60
Respiratory Chest recessions	none	Moderate Costodiaphragmatic	Severe. As 1+ rib and jugular retractions
Auscultatory breath sounds	Vesicular	Wheeze, rales/ronchi	Faint ± severe wheeze ± pronounced rales and rhonci
Skin colour	Normal	Pallor	Cyanosis
General Condition	Not affected	Moderately affected	Severely affected

The clinical score was completed by doctors at inclusion and daily during hospital stay. A clinical core ≥four of ten was required for study inclusion. The score is identical to that used in a study of acute bronchiolitis by Kristjansson et al (1).

Table S2 Background Characteristics of the 404 infants hospitalised with acute bronchiolitis, comparing those attending two- years follow-up and those who did not.

	Attending two-years follow-up n= 294 (72.8)	No two- years follow-up n= 110 (27.2)	p
At birth			
Male sex n (%)	181/294 (61.6)	59/110 (53.6)	0.15
Gestational age (GA) weeks (SD)	38.6 (3.3)	38.8 (2.8)	0.54
Born at GA<37 weeks, n (%)	46/294 (15.6)	14/110 (12.7)	0.46
Birth weight, grams (SD)	3424 (641)	3450 (615)	0.74
At enrolment			
Age, days (range)	124.6 (7,363)	134.6 (10, 364)	0.31
Weight, grams (SD)	6442 (1847)	6692 (1942)	0.23
Eczema n (%)	29/271 (10.7)	11/103 (10.7)	1
One previous episode of wheeze	69/ 260 (26.5)	29/ 102 (28.4)	0.72
Length of hospital stay, hours (SD)	80.3 (66.0)	80.0 (70.9)	0.97
Need of supportive treatment, n (%)	152/294 (51.7)	52/110 (47.3)	0.43
Virus detected during acute bronchiolitis			
RSV, n (%)	219/266 (82.3)	81/97 (83.5)	0.79
HRV, n (%)	93/266 (35.0)	29/97 (29.9)	0.37
HRV A or B, n (%)	28/266 (10.5)	7/97 (7.2)	0.34
HRV C, n (%)	65/266 (24.4)	22/97 (22.7)	0.73
RSV, high genomic load, n (%)	145/266 (54.5)	55/97 (56.7)	0.71
HRV, high genomic load, n (%)	16/266 (6.0)	7/97 (7.2)	0.68
More than 1 virus, n (%)	170/ 266 (63.9)	54/97 (55.7)	0.15
Allergic sensitisation, IgE ≥ 0.35			
Any sensitisation, n (%)	22/271 (8.1)	9/97 (9.3)	0.72
Any food sensitisation, n (%)	20/271 (7.4)	8/97 (8.2)	0.78
Any inhalant sensitisation, n (%)	5/267 (1.9)	2/97 (2.1)	0.91
Egg sensitisation, n (%)	8/271 (3.0)	4/97 (4.1)	0.58
Cow's milk sensitisation, n (%)	13/271 (4.8)	4/97 (4.1)	0.79
Peanut sensitisation, n (%)	3/271 (1.1)	1/97 (1.0)	0.95
Polysensitisation, n (%)	6/268 (2.2)	1/97 (1.0)	0.46
Cortisol geometric mean, mmol/l (95% CI)	42.0 (32.9, 53.7)	35.2 (30.9, 10.0)	
Parental education			
Maternal Education[a] (SD)	3.99 (0.98)	3.47 (0.98)	**<0.001**
Paternal Education[a] (SD)	3.85 (1.0)	3.57 (0.92)	**0.019**
Parental allergic diseases			
Any n (%)	128/258 (49.6)	46/99 (46.5)	0.59
Maternal Asthma, n (%)	36/ 235 (15.3)	12/90 (13.3)	0.65
Paternal Asthma, n (%)	31/235 (13.2)	12/90 (13.3)	0.97
Maternal Rhinoconjungtivitis, n (%)	42/258 (16.3)	20/98 (20.4)	0.36
Paternal Rhinoconjungtivitis, n (%)	56/258 (21.7)	14/98 (14.3)	0.12
Maternal eczema, n (%)	31/263 (11.8)	9/98 (9.2)	0.48
Paternal eczema, n (%)	25/263 (9.5)	4/ 98 (4.1)	0.092
Environment			
Smoking at home n (%)	38/253 (15.0)	20/88 (22.7)	0.097

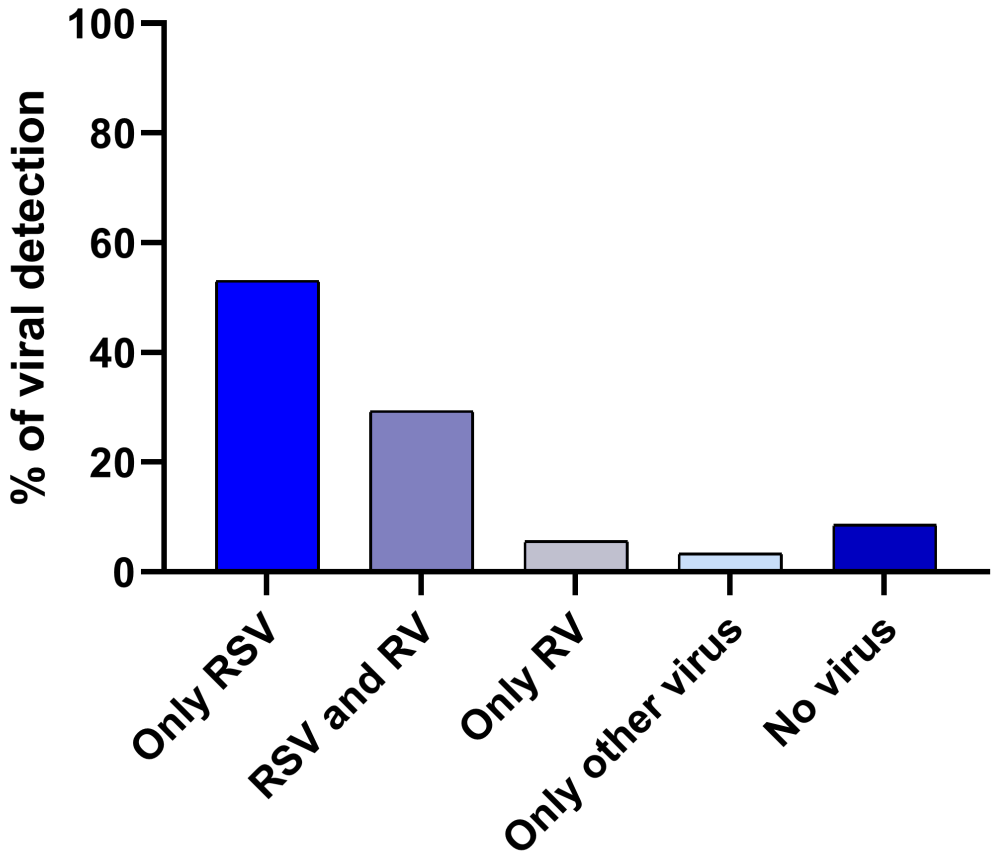
[a] Education was categorised from 1 (no school completed) to 5 (higher education, more than three years)

Table S3 The distribution of low sensitisation defined by IgE 0.10- 0.34 kU/l before one year of age, of the 294 infants hospitalised with bronchiolitis and attending two- year follow up, shown for those with and without recurrent wheeze.

	Recurrent wheeze n= 143 (48.6)	No recurrent wheeze n= 151 (51.4)	p
Low Allergic sensitisation			
Any sensitisation, n (%)	18/130 (13.8)	17/141 (12.1)	0.66
Any food sensitisation, n (%)	18/130 (13.8)	17/141 (12.1)	0.66
Any inhalant sensitisation, n (%)	15/129 (11.6)	14/138 (10.1)	0.70
Egg sensitisation, n (%)	18/130 (13.8)	17/141 (12.1)	0.66
Cow's milk sensitisation, n (%)	15/130 (11.5)	16/141 (11.3)	0.96
Peanut sensitisation, n (%)	15/130 11.5)	15/141 (10.6)	0.81

Fig S1

The figure shows the distribution of the viruses analysed in 266 infants hospitalized with bronchiolitis disease (RSV 53%, HRV and RSV 29.3%, HRV 5.6%, other viruses 3.4%, no virus detected 8.6%)



REFERENSES

1. Kristjansson S, Lodrup Carlsen KC, Wennergren G, Strannegard IL, Carlsen KH. Nebulised racemic adrenaline in the treatment of acute bronchiolitis in infants and toddlers. Archives of disease in childhood. 1993;69(6):650-4.