Virus type and genomic load in acute bronchiolitis: severity and treatment response with inhaled adrenaline

Håvard O. Skjerven1,2, Spyridon Megremis3,4, Nikolaos G. Papadopoulos3,4, Petter Mowinckel2, Kai-Håkon Carlsen1,2, Karin C. Lødrup Carlsen2,1

1Institute of Clinical Medicine, University of Oslo, Oslo, Norway
2Department of Paediatrics, Oslo University Hospital, Oslo, Norway
3Allergy Dept., 2nd Paediatric Clinic, University of Athens, Greece
4Centre for Paediatrics and Child Health, Institute of Human Development, University of Manchester

Address correspondence to: Håvard Ove Skjerven Department of Paediatrics, Ullevål, Oslo University Hospital, Postboks 4956 Nydalen, 0424 Oslo [h.o.skjerven@medisin.uio.no], +4722118765.

Alternative corresponding author: Karin Lødrup Carlsen, Depart Håvard Ove Skjerven Department of Paediatrics, Ullevål, Oslo University Hospital, Postboks 4956 Nydalen, 0424 Oslo [k.c.l.carlsen@medisin.uio.no], +4722118765.

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

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Contributors’ Statement

Håvard Ove Skjerven: Dr. Skjerven conceptualized and designed the study, coordinated and supervised data collection, analysed data, drafted the initial manuscript and approved the final manuscript as submitted.

Spyridon Megremis and Nikolaos G. Papadopoulos: Dr. Megremis and Dr. Papadopoulos carried out the viral analyses and interpretation of data, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Petter Mowinckel: Mr. Mowinckel carried out the statistical analyses and interpretation of data, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Kai-Håkon Carlsen: Dr Carlsen conceptualized and designed the study, analysed data, reviewed and revised the manuscript and approved the final manuscript as submitted.

Karin Cecilie Ødrup Carlsen: Dr Carlsen has been the PI of the study, conceptualized and designed the study, analysed data, reviewed and revised the manuscript and approved the final manuscript as submitted.

All authors have full insight into all data

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A special thank goes to the patients and parents participating in the study.
Abstract

Background

Acute bronchiolitis frequently causes infant hospitalization. Studies on different viruses or viral genomic load and disease severity or treatment effect are conflicting. We aimed to investigate if airway viruses were associated with disease severity or the treatment effect of inhaled adrenaline.

Methods

Nasopharyngeal aspirates were collected in 363 infants with acute bronchiolitis in an RCT that compared inhaled racemic adrenaline versus saline. Virus genome was identified and quantified by PCR analyses. Severity was assessed by the length of stay (LOS) and the use of supportive care.

Results

Respiratory syncytial virus (RSV) (83%) and Human rhinovirus (34%) was most commonly detected. Seven other viruses were present in 8-15% of the patients. Two viruses or more (maximum seven) were detected in 61% of the infants. Virus type or confection was not associated with disease severity. A high genomic load of RSV was associated with a longer LOS and increased use of oxygen and ventilatory support. Treatment effect of inhaled adrenaline was not modified by virus type, load or coinfection.

Discussion

In infants hospitalized with acute bronchiolitis, disease severity was not associated with specific viruses or the total number of viruses detected. High genomic load of RSV was associated with more severe disease.
**Introduction**

Acute bronchiolitis in infants is a major health burden worldwide, closely associated with seasonal epidemics of RSV (respiratory syncytial virus)[1, 2]. Detection of more than one virus has been reported in up to 40% of the infants[3]. Modern techniques produce increasing detection rates of both RSV and other viruses with a less certain role in the causation of acute bronchiolitis[4].

Treatment of acute bronchiolitis in hospitalized infants is generally supportive[5], although bronchodilators, including inhaled adrenaline, are commonly used.[6, 7] We recently showed in 404 infants that treatment with inhaled racemic adrenaline was not superior to inhaled isotonic saline[8]. Longitudinal studies have shown that many, but not all children with acute bronchiolitis develop asthma later[9], and that human rhinovirus (HRV) more than RSV in children hospitalized for obstructive airways disease increases the risk of later asthma[10, 11]. However, it is not clear if HRV represents a marker of predisposition to obstructive airways disease or has a causative role. Studies to assess if viral aetiology may modify treatment effect with inhaled bronchodilators have been requested[12], but not previously been reported.

Attempts have been made to link the presence of different viruses with severity of disease, with conflicting results in regards to RSV[12-16] and HRV, including subtypes[17, 18]. RSV has been associated with increased disease severity in some[12-14], but not all[3, 15] studies. Studies have shown higher[15], unchanged[16] or lower[12, 18-20] clinical severity in patients with a positive compared to a negative finding of HRV. The presence of the recently discovered[21] HRV type C strains has been associated with more severe obstructive airways disease in young children with an acute lower airway infection[22, 23]. However, studies focusing on infants with bronchiolitis have been unclear on this association[18, 20].
Studies of viral genomic load in nasopharyngeal aspirate have shown a positive relationship with disease severity, as a higher concentration of RSV virus in nasopharyngeal aspirates has been associated with a more severe disease [3, 24-28]. The same association has not been found in regards to HRV. [29]

The significance of coinfections, found in 9 to 41% of patients, may have major clinical impact on the guidelines for isolation of hospitalized patients. However, studies have shown conflicting association with disease severity[3, 19, 30]. Brand et al[3] examined 142 samples for 15 different viruses, found more than one virus in 41%, but no association with disease severity. In contrast, Richard et al[30] found that coinfections (24%, including RSV, Rhinovirus and six other viruses) increased the risk of PICU admission 2.7 times. The aims of the present study were to investigate if the presence or concentration of individual or multiple viruses were associated with disease severity in acute bronchiolitis. Secondarily, we aimed to evaluate if detected viruses modified the response to inhaled racemic adrenaline.

**Subjects and Methods**

**Study design**

The Bronchiolitis ALL-SE study is a multicentre, randomized, double-blinded, factorial designed clinical trial comparing the effect of inhaled racemic adrenaline versus saline and two inhalation strategies (on demand versus fixed schedule) (Figure 1) in infants in Norway in two consecutive winter seasons from January 2010 through May 2011[8]. Inclusion criteria were age <12months and clinical signs of moderate to severe bronchiolitis. An overall clinical score of 4 or higher (on a scale of 0 to 10, with higher scores indicating more severe illness (see Table E1) were used as inclusion criteria, indicating moderate to severe illness. 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 four weeks; and any glucocorticoid therapy in the preceding four weeks.

Patients were enrolled at all hours, limited occasionally by the overall capacity of the acute ward. 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 Bio bank 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. The trial was registered in ClinicalTrials.gov (NCT00817466) and EudraCT (2009-012667-34).

Subjects

The present study included the 363 infants with nasopharyngeal aspirate available, out of the total 404 infants hospitalized with moderate to severe acute bronchiolitis that participated in a randomized controlled trial on inhalation treatment [8] (62% boys, mean age 4.2 months) in one of the eight participating hospitals in South East Norway. Median length of stay (LOS) was 67.1 (95% CI 58.4–71.3) hours. Baseline characteristics were comparable in the treatment groups (Table 1) and in the patients with and without available samples of nasopharyngeal aspirates.

Methods

Details on randomization and study medication are described elsewhere[8].

The use of supportive care was recorded daily and verified in manual patient record reviews.
The LOS was defined as the time from the first study inhalation until discharge from the hospital, as recorded in the medical record for each patient.[8] Most children were discharged between 8 a.m. and 11 p.m.

Nasopharyngeal aspirates were collected using a standardized procedure, performed by trained paediatric nurses at inclusion of the trial, with a tracheal suction set (Unomedical A/S, Lejre, Denmark), immediately frozen at -20°C and transferred, without melting, to central storage in Oslo University Hospital (-78°C) within four weeks. Each sample was melted and separated into two portions, one of which was transported on dry ice and batch analysed at the Allergy department of the University of Athens. Several of the participating hospitals, including in 80% of the patients at Oslo University Hospital, performed additional nasopharyngeal sampling and viral analyses by PCR and immunoassays as part of their local routines.

Virus nucleic acids were isolated using the QIAamp Viral RNA Mini Kit (Qiagen, Limburg, Netherlands) and carrier RNA (Qiagen) for increased isolation yield of small sequences. Reverse transcriptase PCR (RT-PCR) was performed using SuperScript® II Reverse Transcriptase (Invitrogen, Life Technologies, Carlsbad, CA, USA) with a starting volume of 10µl of genetic material in a 20µl final reaction volume with default reaction conditions (Invitrogen).

Amplification of viral target sequences was performed using dual priming oligonucleotide (DPO) and real amplicon amplification (READ) technology (Magicplex RV Panel Real-time Test, Seegene, Eschborn, Germany)[31]. The assay allows the detection of Influenza A (FluA, including H5N1, H1N1) virus, Influenza B (FluB) virus, Respiratory Syncytial virus A/B (RSV), Metapneumovirus (MPV), Adenovirus (AdV, B/C/E and some of A/D/F), Coronavirus (CoV, 229E/NL63/OC43), Human Rhinovirus (HRV, A/B/C)- HEV, Human
Bocavirus (HBoV, 1/2/3/4) and Parainfluenza virus (PIV, 1/2/3/4). Positive HRV samples were subsequently subtyped for HRV A, B and C based on the published PCR-based assay by Wisdom et al. [32] The assay includes three internal controls; a nucleic acid isolation and RT amplification control against the Human RNAse P sequence, and two virus detection controls (positive/negative). PCR reactions were performed in the Rotorgene Q 6plex Real-time PCR platform (Qiagen) and the results were analysed in the Seegene Viewer for Real-time instruments (Seegene).

A real-time PCR reaction was considered positive when the accumulation of fluorescent signal crosses the cycle threshold (Ct), that is, the signal strength required for a detection to be identified. In the assay that we used, the cycle threshold for a virus should be crossed before the 20th cycle of amplification, for a sample to be considered positive for this specific virus, as determined by the manufacturer.

Our protocol included two normalization steps:

i. Evaluation of the RNA isolation and RT-PCR efficiencies: 95% of the RNA/RT-PCR control Cts followed normal distribution over a very small range of Cts (2-3 cycles). Samples with poor RNA isolation/RT-PCR amplification efficiencies (high Cts) were selected and excluded from the analysis as outliers of the bench-protocol (21 samples) since they could heavily bias the clustering procedure;

ii. Normalization of vira-specific Cts against the EPC (extraction and PCR control - virus positive control); this normalization was the equation: delta Ct= Ct_{target}-Ct_{EPC}, thus defining the delta Ct which allows the comparison of same viral sequences between different samples. Samples with low delta Ct values represent PCR reactions with high genomic load for the specific target viral sequence and high delta Ct-values represents a low genomic load, correspondingly. As the Ct-values represent very different actual number of microbes for the
different virus types, semi-quantitative categorization into tertiles or quartiles is common. [28] However, as the different viruses may show a variety of Ct-patterns, researcher-driven categorization may be arbitrary and introduce cut-offs that don’t harmonize with the natural distribution of viral concentrations. We therefore chose a data-driven approach with the application of cluster analyses in order to improve classification of viral genomic load.

Based on the PCR threshold cycles (0-20), the algorithm iteratively estimated the cluster means and assigns each sample to the cluster whose mean is closest to this particular sample. After all of the samples were assigned to clusters, the cluster centers were recomputed, until no center changed appreciably or the maximum number of iterations (ten) was reached. A preset number of clusters were set to maximum five.

Complementary analyses on all outcomes were performed on samples positive versus negative for the highest concentration clusters only. In the present paper, analyses were done for high versus all other clusters (Figure E1). The clusters are hereafter referred to as genomic load, and analyses were performed for high versus all other groups of genomic load.

**Outcomes**

Disease severity was assessed by the LOS and the level of supportive care, categorized as 1) (no supportive care), 2) (use of oxygen and/or nasogastric tube feeding) or 3) (use of ventilatory support) as previously published by Brand et al[3].

The outcome for modification of treatment effect by the presence of virus was given by the interaction between the presence of virus (RSV, HRV or multiple viruses) and randomization group (inhaled racemic adrenaline/saline) on LOS.

**Statistical analyses**

Continuous data are presented as means (95% confidence intervals), and categorical data are presented as numbers and percentages. Categorical data were analysed with the use of the
Pearson chi-square test. Because data on LOS and level of supportive care had a non-normal distribution, comparisons between groups were analysed with the use of a robust, two-sample t-test and Huber’s M-estimator, with 95% confidence intervals. This method was also used to analyse the association between virus subgroups and the level of supportive care (0-3) as an ordinal outcome. Holm’s sequentially rejective multiple test procedure was applied[33].

Analysis of viral aetiology as an effect modifier of treatment was performed by interaction analyses in a robust linear regression model for the main outcome (LOS).

The level of significance was set at 0.05. Analyses were performed with the use of SPSS version 21.1 (cluster analyses) and Stata software, version 13.1.

Results

One or more viruses were detected in 91% of the patients, with RSV (83%) and HRV (34%) as the most common. Other airway viruses were detected at lower frequencies, ranging from the identification of influenza B in 8% of the population to adenovirus and coronavirus, each identified in 15% of study subjects. Two or more viruses were detected in 61%, three or more viruses in 30%, and up to seven viruses were simultaneously detected (Figure 2). RSV accounted for 82% (89/108) of the monoinfections. See online supplement (Figure E2 and table E2) for details. Infants with RSV monoinfection were significantly younger, and infants infected with HRV monoinfection were significantly older, than infants with other detected viruses (Table E1).

One or more viruses were found in high genomic load in 70% of the patients. RSV was found in 55% of the study population, HRV in 6% and other viruses in 1-12% of the cases (Figure E1), co-infection in 18%; three or more viruses in 3% and up to four viruses were
RSV was present in 81% (36/188) of the patients with one virus detected only. See online supplement (Figure E1 and table E3) for details.

Neither LOS or the level of supportive care were associated with the presence of RSV, HRV A/B, HRV C, influenza A, influenza B, parainfluenzavirus, adenovirus or bocavirus (Table E2). Although LOS was significantly longer in the presence of coronavirus (17.1 hours (95% confidence interval (CI) 1.2 to 33.0 hours, p=0.04), and shorter with the detection of human metapneumovirus (-19.3 hours (CI -36.0 to -2.5, p=0.02)) (Figure 3), the results were no longer statistically significant after adjusting for multiple testing.

However, the presence of RSV in high viral genomic load was associated with the severity of disease in terms of longer LOS and higher level of supportive care (significantly more use of oxygen and ventilatory support) (Table E3). No other virus classified with a high genomic load was associated with the severity of disease.

Neither the presence of coinfections in general, the specific combination of HRV and RSV, or having one virus only (mono-infection) were associated with disease severity (Table E2, Figure 3). However, in the analyses of high genomic load only, mono-infection of RSV was associated with an increased level of supportive care (Table E3).

Treatment response of inhaled racemic adrenaline in terms of LOS was not modified by the presence of RSV or HRV or ≥2 viruses detected (all p>0.40) (Figure E3) in regular or high viral genomic load analyses.

Discussion
In infants with acute bronchiolitis, we found no association between the presence of one or combinations of viruses (RSV and HRV, RSV only, no virus or ≥2 viruses) and disease
severity after adjustment for multiple testing. A high genomic viral load for RSV only, but not for any of the other viruses or combinations thereof was associated with increased LOS and more use of oxygen and ventilatory support. We found no associations between the presences of single or multiple airways viruses, including RSV and HRV and the treatment effect of inhaled racemic adrenaline versus saline inhalations.

The high rate (91%) of virus detection, including RSV in 83% and coinfections in 61% is in line with a recent study from Finland (86% detection rate) of children with bronchiolitis,[34] but is high compared to other studies[3, 19, 30] (up to 41% coinfections). [1, 13] Interestingly, the Finnish study with a high identification rate of at least one virus reported only 15% of children with more than one virus,[34] whereas in the same multicentre study including also 16 American study sites and of 2615 children <2 years of age with severe bronchiolitis, RSV was detected in 67%, with additional viruses detected in 31%.[28] Further, approximately half of the prematurely born infants younger than 6 months of age in Brazil with lower respiratory tract infection and one identified virus had coinfection with at least one virus, most commonly RSV and HRV.[35] Similarly to Brand et al, [3], but in contrast to Marguet et al, [19] we found that infants with RSV monoinfection were significantly younger than infants with coinfections. However, unlike Marguet et al, [19] we found that infants with HRV monoinfection were significantly older than infants with all other detected viruses except for bocavirus. These apparent discrepancies may be related to study design as the mean age was lower (2.4 months) and infants with previous wheeze were excluded in the latter study. [19]

Coinfections with up to seven viruses as well as 30% of children infected with three viruses or more have to our knowledge not previously been published. The differences in virus detection may be in part explained by modern and highly sensitive virus detection techniques, a relatively homogenous the study population of moderately to severely ill infants with a strict definition of acute bronchiolitis and the use of a structured nasopharyngeal aspirate procedure
performed by trained and experienced personnel. Also, a large proportion (77% of one- to
two-year olds, 96% of three- to five-year-olds) of young Norwegian children attend day-care
centres[36], which might increase the incidence and morbidity of respiratory virus
infections[37] that subsequently infect their infant siblings.

Our analyses indicated a longer LOS with coronavirus and shorter LOS with
metapneumovirus, in line with a previous report[19]. However, we found no significant
association between type of virus or coinfection (with two to seven viruses) and disease
severity or LOS after adjusting for multiple testing in the present study. This is in contrast to
previous reports of small sample sized studies [30, 38], as well as in 61 premature infants with
severe LRTI in Brazil,[35] RSV combined with HRV was associated with increased LOS
compared to RSV alone. Our findings are, however, in line with a recent, large study that
found no association between disease severity and coinfections among the 31% of infants
with RSV and another virus[28] . The clinical relevance of the presence of viruses known to
be pathogenic, including Flu[40], AdV[41], HBoV[42], CoV[43], PIV[44] and MPV[45],
found in significant rates in our study population (n=37-56, 10-15%) are not clear.

Our novel approach with the use of cluster analyses to increase specificity showed that high
genomic RSV load, but not HRV load, was associated with an increased severity of disease,
in line with previous reports[3, 24-27, 29] and a recent report including more than 2000
children using a tertile approach to categorize genomic RSV load. [28] Cluster analysis has
the advantage that it can take into account the pattern of genomic load individually for each
virus. The presence of high genomic load of RSV and HRV in combination was also
associated with significantly increased LOS, in line with the findings in premature infants in
Brazil. However, we were not able to investigate if this was due to coinfection or mainly
related to the high genomic load
In the present study, the percentage of children with more than one virus (coinfection) decreased from 61% to 18% by considering high genomic load only. Identification of viruses does not necessarily infer causal associations with bronchiolitis, as suggested by the present study. However, the very high rate of RSV, very low rates of mono-infections with viruses other than RSV and lack of association between HRV (A/B or C) or multiple viruses detected with disease severity, support the dominating role of RSV in acute bronchiolitis, which is further supported by the association between high concentrations of RSV and disease severity.

Although similar detection rates of HRV in young children have previously been found regardless of the presence of a symptomatic airway infection, as recently Yoshida et al showed, comparing hospitalized patients (mean age 1.5 years), finding HRV in 35% of controls versus in 29% of patients, while RSV was detected in 3% of controls versus 39% of patients[4], the role of HRV C has been unclear. Bizzintino et al found an association between HRV C and severity of acute asthma[23] in children >two years of age, while Cox et al[46] found increased hospital admissions in 197 children <five (mean age 31.0 months) years of age. We were not able to identify studies in hospitalized infants, assessing disease severity, of sufficient power.

The viral genomic load should be compared to later obstructive airways disease in follow-up studies. Nevertheless, we found no significant impact of viral genomic load on treatment effect of inhaled racemic adrenaline.

The present study found no evidence for effect modification of inhaled racemic adrenaline by RSV, HRV or multiple viruses detected, neither in regular or high specificity analyses. This is in line with our previous report that showed no effect of inhaled racemic adrenaline in the whole study population[8]. Mansbach et al[12] suggested in 2010 that future clinical trials should categorize results by infectious pathogen, including HRV, as such information was
lacking. Our negative result does not support the use of bronchodilators to be guided by viral aetiology.

**Limitations**

The present study population was insufficient for robust subgroup analyses for all types of viruses. However, it is likely that analyses of the major viruses, RSV and HRV are sufficiently large to indicate the lack of clinically relevant associations.

Although interaction tests are considered appropriate for detecting effect modification, these analyses generally requires four times the population size to detect significant differences of similar magnitude.[47] However, the study population in the present study is large, and we have a high detection rate of the major viruses. Nevertheless the lack of significant interaction must be interpreted with caution.

**Conclusion**

In acute bronchiolitis in infants, coinfection of respiratory viruses was found in 61% of the patients, with up to seven viruses simultaneously detected. Disease severity was not associated with the identification of virus or the total number of viruses detected, but a high genomic load of RSV was associated with a longer LOS and more use of oxygen and ventilatory support. Neither the presence of viruses, nor the viral genomic load modified the treatment response to inhaled adrenaline.
References


Table and figures

Legends

Table 1: Baseline characteristics of 363 infants with acute bronchiolitis are given according to treatment group of inhaled racemic adrenaline or inhaled saline

*Parental reported previously diagnosed allergy in interview with physician at inclusion.
†coughing, rattling or respiratory distress
‡ 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.

Figure 1: Randomization of the study patients.
In one patient, the study medication was discontinued due to administrative failure, as there was insufficient supply of study medication available.

Figure 2: Frequencies of patients by simultaneously detected number of viruses

Figure 3: Mean length of stay for 363 infants with acute bronchiolitis grouped by individual viruses and viral subgroups
Pairwise comparisons are shown for a positive (top) versus a negative (corresponding below) PCR for each virus or viral subgroups. The estimated mean length of stay was adjusted for age in a robust linear regression model. The associations between type/subgroups of virus and length of stay were no longer statistically significant after adjustment for multiple comparisons.
Tables

Table 1: Baseline characteristics of 363 infants with acute bronchiolitis are given according to treatment group of inhaled racemic adrenaline or inhaled saline

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Adrenaline (n=184)</th>
<th>Saline (n=179)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex - no. (%)</td>
<td>62.0</td>
<td>58.1</td>
</tr>
<tr>
<td>Age, months (95% CI)</td>
<td>4.2 (3.7-4.6)</td>
<td>4.2 (3.8-4.6)</td>
</tr>
<tr>
<td>Father Caucasian</td>
<td>92.5%</td>
<td>90.9%</td>
</tr>
<tr>
<td>Mother Caucasian</td>
<td>90.8%</td>
<td>91.7%</td>
</tr>
<tr>
<td>Atopic eczema</td>
<td>9.9%</td>
<td>10.8%</td>
</tr>
<tr>
<td>Reported allergies*</td>
<td>1.8%</td>
<td>1.8%</td>
</tr>
<tr>
<td>1 previous BO</td>
<td>25.8%</td>
<td>30.1%</td>
</tr>
<tr>
<td>&gt;1 week of persistent respiratory symptoms†</td>
<td>12.1%</td>
<td>14.7%</td>
</tr>
<tr>
<td>Parental asthma</td>
<td>22.6%</td>
<td>25.9%</td>
</tr>
<tr>
<td>Parental rhino conjunctivitis</td>
<td>30.5%</td>
<td>32.1%</td>
</tr>
<tr>
<td>Clinical score‡ (95% CI)</td>
<td>4.9 (4.8-5.1)</td>
<td>4.9 (4.7-5.0)</td>
</tr>
<tr>
<td>SpO2(95% CI)</td>
<td>96.0 (95.5-96.5)</td>
<td>96.0 (95.5-96.5)</td>
</tr>
<tr>
<td>Respiratory rate (95% CI)</td>
<td>53.3 (51.6-55.0)</td>
<td>54.0 (52.3-55.7)</td>
</tr>
<tr>
<td>Heart rate (95% CI)</td>
<td>154.6 (151.9-157.3)</td>
<td>151.5 (148.7-154.3)</td>
</tr>
</tbody>
</table>

*Parental reported previously diagnosed allergy in interview with physician at inclusion.
†coughing, rattling or respiratory distress
‡ 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.
404 infants hospitalized for acute bronchiolitis

363 nasopharyngeal aspirate obtained

184 assigned to inhaled adrenaline

179 assigned to inhaled saline

29 discontinued study:
- 21 treatment failure
- 2 side-effects
- 6 parental wish
- 0 administrative failure

155 completed inhaled adrenaline treatment

140 completed inhaled saline treatment

295 completed treatment
A

Respiratory Syncytial Virus
Rhinovirus
Rhinovirus A/B
Rhinovirus C
Influenza A
Influenza B
Parainfluenza Virus
Metapneumovirus
Adenovirus
Coronavirus
Bocavirus

Length of stay (hours)

p=0.02
p=0.04
Online Supplement

Virus type and genomic load in acute bronchiolitis: severity and treatment response with inhaled adrenaline

Short title: Viral genomic load and bronchiolitis

Håvard O Skjerven, M.D.1,2; Spyridon Megremis, Ph.D.3,4, Nikolaos G. Papadopoulos3,4, M.D., Ph.D., Petter Mowinckel M.Sc.2, Kai-Håkon Carlsen M.D., Ph.D., 1,2, Karin C. Lødrup Carlsen M.D., Ph.D.2,1

Affiliations:

1. Institute of Clinical Medicine, University of Oslo, Oslo, Norway
2. Department of Paediatrics, Oslo University Hospital, Oslo, Norway
3. Allergy Dept., 2nd Paediatric Clinic, University of Athens, Greece
4. Centre for Paediatrics and Child Health, Institute of Human Development, University of Manchester

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

Address correspondence to: Håvard Ove Skjerven Department of Paediatrics, Ullevål, Oslo University Hospital, Postboks 4956 Nydalen, 0424 Oslo [h.o.skjerven@medisin.uio.no], +4722117663.
Tables

Table E1: Frequencies and use of supportive care in infants with acute bronchiolitis grouped by viral identification

Analyses of differences between subgroups are adjusted for age in logistic regression for categorical outcomes or robust linear regression for continuous outcomes. Differences are not statistically significant except otherwise noted.

*p=0.04.

Table E2: Frequencies, use of supportive care and LOS by viral identification in the lowest sensitivity (highest concentration) clusters.

* p=0.006, † p=0.021, ‡ p=0.004 adjusted for age in logistic regression.§ p=0.003, ** p=0.042, †† p=0.004, ‡‡ p=0.046, in robust linear regression adjusted for age (estimated means)
Table E1: Frequencies and use of supportive care in infants with acute bronchiolitis grouped by viral identification

<table>
<thead>
<tr>
<th>Viral subgroup</th>
<th>N</th>
<th>Age, months (95% CI)</th>
<th>SpO2 at inclusion</th>
<th>Clinical score at inclusion</th>
<th>Nasogastric tube feeding</th>
<th>Oxygen support</th>
<th>Ventilatory support</th>
<th>Level of supportive care</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>363</td>
<td>4.2 (3.9-4.5)</td>
<td>96.0 (95.6-96.3)</td>
<td>4.9 (4.8-5.0)</td>
<td>106/359 (30%)</td>
<td>151/342 (44%)</td>
<td>27/363 (7%)</td>
<td>1.59 (1.52-1.65)</td>
</tr>
<tr>
<td>No detectable virus</td>
<td>33(9%)</td>
<td>3.4 (2.5-4.4)</td>
<td>95.9 (94.5-97.2)</td>
<td>4.8 (4.5-5.1)</td>
<td>10/33 (30%)</td>
<td>16/31 (52%)</td>
<td>2/33 (6%)</td>
<td>1.61 (1.36-1.79)</td>
</tr>
<tr>
<td>Influenza A</td>
<td>39 (11%)</td>
<td>5.1 (4.1-6.1)</td>
<td>97.0 (96.3-97.6)</td>
<td>4.9 (4.6-5.3)</td>
<td>13/37 (35%)</td>
<td>13/38 (34%)</td>
<td>2/39 (5%)</td>
<td>1.49 (1.29-1.68)</td>
</tr>
<tr>
<td>Influenza B</td>
<td>30 (8%)</td>
<td>4.5 (3.4-5.6)</td>
<td>96.1 (95.1-97.2)</td>
<td>5.0 (4.7-5.4)</td>
<td>14/30 (47%)</td>
<td>17/30 (57%)</td>
<td>2/30 (7%)</td>
<td>1.73 (1.52-1.95)</td>
</tr>
<tr>
<td>Respiratory syncytialvirus</td>
<td>300 (83%)</td>
<td>4.2 (3.9-4.5)</td>
<td>96.0 (95.6-96.4)</td>
<td>4.9 (4.8-5.0)</td>
<td>89/297 (30%)</td>
<td>126/282 (45%)</td>
<td>25/300 (8%)</td>
<td>1.61 (1.54-1.68)</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>49 (13%)</td>
<td>4.1 (3.2-5.0)</td>
<td>96.0 (95.1-96.9)</td>
<td>4.9 (4.6-5.2)</td>
<td>9/48 (19%)</td>
<td>18/48 (38%)</td>
<td>3/49 (6%)</td>
<td>1.45 (1.27-1.62)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>55 (15%)</td>
<td>4.6 (3.9-5.4)</td>
<td>95.1 (94.0-96.2)</td>
<td>5.1 (4.7-5.4)</td>
<td>16/54 (30%)</td>
<td>25/51 (49%)</td>
<td>3/55 (6%)</td>
<td>1.58 (1.42-1.74)</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>56 (15%)</td>
<td>4.4 (3.5-5.2)</td>
<td>96.7 (96.0-97.4)</td>
<td>5.0 (4.7-5.4)</td>
<td>19/56 (34%)</td>
<td>25/52 (48%)</td>
<td>5/56 (9%)</td>
<td>1.63 (1.45-1.80)</td>
</tr>
<tr>
<td>Human rhinovirus (all)</td>
<td>122 (34%)</td>
<td>4.7 (4.1-5.2)</td>
<td>96.0 (95.4-96.6)</td>
<td>4.9 (4.8-5.1)</td>
<td>38/121 (31%)</td>
<td>46/115 (40%)</td>
<td>9/122 (7%)</td>
<td>1.56 (1.45-1.68)</td>
</tr>
<tr>
<td>Human rhinovirus A/B</td>
<td>35 (10%)</td>
<td>4.2 (3.1-5.3)</td>
<td>95.5 (94.0-97.0)</td>
<td>5.0 (4.6-5.3)</td>
<td>11/34 (32%)</td>
<td>13/34 (38%)</td>
<td>3/35 (9%)</td>
<td>1.60 (1.38-1.82)</td>
</tr>
<tr>
<td>Human rhinovirus C</td>
<td>87 (24%)</td>
<td>4.8 (4.2-5.5)</td>
<td>96.3 (95.6-96.9)</td>
<td>4.9 (4.7-5.1)</td>
<td>27/87 (31%)</td>
<td>33/81 (41%)</td>
<td>6/87 (7%)</td>
<td>1.55 (1.42-1.68)</td>
</tr>
<tr>
<td>Human bocavirus</td>
<td>37 (10%)</td>
<td>5.5 (4.3-6.6)</td>
<td>95.1 (93.5-96.7)</td>
<td>4.8 (4.5-5.1)</td>
<td>10/37 (27%)</td>
<td>16/36 (44%)</td>
<td>1/37 (3%)</td>
<td>1.57 (1.38-1.75)</td>
</tr>
<tr>
<td>Parainfluenzavirus</td>
<td>48 (13%)</td>
<td>4.4 (3.6-5.2)</td>
<td>96.5 (95.5-97.4)</td>
<td>5.0 (4.7-5.3)</td>
<td>9/46 (20%)</td>
<td>17/48 (35%)</td>
<td>1/48 (2%)</td>
<td>1.42 (1.26-1.57)</td>
</tr>
<tr>
<td>&gt;1 virus</td>
<td>223 (61%)</td>
<td>4.5 (4.1-4.9)</td>
<td>96.0 (95.5-96.4)</td>
<td>4.9 (4.8-5.1)</td>
<td>64/220 (29%)</td>
<td>88/210 (42%)</td>
<td>17/223 (8%)</td>
<td>1.57 (1.49-1.65)</td>
</tr>
<tr>
<td>Respiratory syncytialvirus only</td>
<td>89 (25%)</td>
<td>3.3 (2.9-3.9)</td>
<td>96.1 (95.4-96.7)</td>
<td>4.9 (4.7-5.0)</td>
<td>28/89 (31%)</td>
<td>43/84 (51%)</td>
<td>8/89 (9%)</td>
<td>1.70 (1.56-1.83)</td>
</tr>
<tr>
<td>Human rhinovirus only</td>
<td>9 (2%)</td>
<td>6.3 (4.4-8.1)</td>
<td>95.6 (93.6-97.5)</td>
<td>4.4 (3.7-5.2)</td>
<td>1/9 (11%)</td>
<td>2/9 (22%)</td>
<td>0/9 (0%)</td>
<td>1.33 (0.95-1.72)</td>
</tr>
<tr>
<td>Respiratory syncytialvirus and</td>
<td>105 (29%)</td>
<td>4.6 (4.0-5.2)</td>
<td>96.1 (95.4-96.8)</td>
<td>5.0 (4.8-5.2)</td>
<td>34/104 (33%)</td>
<td>41/98 (42%)</td>
<td>9/105 (9%)</td>
<td>1.59 (1.47-1.72)</td>
</tr>
<tr>
<td>Human rhinovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
All continuous variables are displayed as means (95% confidence intervals). Analyses of differences between subgroups are adjusted for age in logistic regression for categorical outcomes or robust linear regression for continuous outcomes. Differences are not statistically significant except otherwise noted.
<table>
<thead>
<tr>
<th>Viral subgroup</th>
<th>N</th>
<th>Age, months (95% CI)</th>
<th>SpO2 at inclusion</th>
<th>Clinical score at inclusion</th>
<th>Nasogastric tube feeding</th>
<th>Oxygen support</th>
<th>Ventilatory support</th>
<th>Level of supportive care</th>
<th>Length of Stay (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>363</td>
<td>4.2 (3.9-4.5)</td>
<td>96.0 (95.6-96.3)</td>
<td>4.9 (4.8-5.0)</td>
<td>106 (359) (30%)</td>
<td>151/342 (44%)</td>
<td>27/563 (7%)</td>
<td>1.59 (1.52-1.65)</td>
<td>80.6 (73.8-87.3)</td>
</tr>
<tr>
<td>No virus with high load</td>
<td>110</td>
<td>4.2 (3.6-4.7)</td>
<td>96.3 (95.8-96.8)</td>
<td>4.8 (4.6-4.9)</td>
<td>32/109 (29%)</td>
<td>41/104 (39%)</td>
<td>5/110 (5%)</td>
<td>1.54 (1.43-1.65)</td>
<td>90.6 (77.1-104.1)</td>
</tr>
<tr>
<td>Influenza A</td>
<td>4 (1%)</td>
<td>5.5 (1.2-9.9)</td>
<td>97.3 (93.7-100)</td>
<td>5.0 (3.2-6.8)</td>
<td>1/3 (33%)</td>
<td>0/3</td>
<td>0/4</td>
<td>1.25 (0.45-2.05)</td>
<td>87.8 (31.6-144.0)</td>
</tr>
<tr>
<td>Influenza B</td>
<td>4 (1%)</td>
<td>3.8 (0.2-7.4)</td>
<td>93.4 (87.6-99.9)</td>
<td>5.3 (3.7-6.8)</td>
<td>1/4 (25%)</td>
<td>2/4 (50%)</td>
<td>0/4</td>
<td>1.50 (0.58-2.42)</td>
<td>78.5 (22.8-134.2)</td>
</tr>
<tr>
<td>Respiratory syncytial</td>
<td>200</td>
<td>4.0 (3.6-4.4)</td>
<td>95.7 (95.2-96.2)</td>
<td>5.0 (4.9-5.2)</td>
<td>65/199 (33%)</td>
<td>95/186 (51%) ‡</td>
<td>21/200 (11%)</td>
<td>1.69 (1.59-1.77)†</td>
<td>96.0 (84.9-107.2)‡</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>21</td>
<td>3.9 (2.7-5.1)</td>
<td>96.2 (94.9-97.5)</td>
<td>4.8 (4.4-5.1)</td>
<td>4/21 (19%)</td>
<td>7/20 (35%)</td>
<td>1/21 (5%)</td>
<td>1.48 (1.20-1.75)</td>
<td>70.6 (45.5-95.7)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>43</td>
<td>4.6 (3.7-5.5)</td>
<td>95.0 (93.6-96.3)</td>
<td>5.1 (4.7-5.5)</td>
<td>13/42 (31%)</td>
<td>19/41 (46%)</td>
<td>1/43 (2%)</td>
<td>1.53 (1.37-1.70)</td>
<td>86.7 (67.6-1059)</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>10</td>
<td>4.6 (2.0-7.1)</td>
<td>96.8 (94.3-99.2)</td>
<td>5.2 (4.1-6.3)</td>
<td>3/10 (30%)</td>
<td>4/10 (40%)</td>
<td>0/10</td>
<td>1.50 (1.12-1.88)</td>
<td>99.1 (63.0-135.1)</td>
</tr>
<tr>
<td>Human rhinovirus (all)</td>
<td>23</td>
<td>6.5 (4.7-8.3) ‡‡</td>
<td>95.6 (93.7-97.5)</td>
<td>5.0 (4.6-5.3)</td>
<td>6/23 (26%)</td>
<td>10/22 (45%)</td>
<td>1/23 (4%)</td>
<td>1.48 (1.22-1.73)</td>
<td>107.0 (80.2-133.7)</td>
</tr>
<tr>
<td>Human bocavirus</td>
<td>3 (1%)</td>
<td>9.1 (2.9-15.3) ‡ (p=0.003)</td>
<td>93.3 (77.6-109)</td>
<td>4.0</td>
<td>0/3</td>
<td>2/3 (67%)</td>
<td>0/3</td>
<td>1.67 (0.23-3.10)</td>
<td>102.9 (36.6-169.2)</td>
</tr>
<tr>
<td>Pneumovirus</td>
<td>17</td>
<td>3.8 (2.5-5.1)</td>
<td>97.0 (95.7-98.3)</td>
<td>4.8 (4.3-5.3)</td>
<td>4/16 (25%)</td>
<td>7/16 (44%)</td>
<td>0/17</td>
<td>1.41 (1.15-1.67)</td>
<td>79.9 (52.1-107.7)</td>
</tr>
<tr>
<td>&gt;1 virus</td>
<td>67</td>
<td>4.7 (4.0-5.5)</td>
<td>95.2 (94.2-96.2)</td>
<td>5.1 (4.9-5.4)</td>
<td>20/86 (30%)</td>
<td>33/63 (52%)</td>
<td>2/67 (3%)</td>
<td>1.58 (1.45-1.72)</td>
<td>99.4 (82.9-116.1)</td>
</tr>
<tr>
<td>Respiratory syncytial</td>
<td>145</td>
<td>3.8 (3.4-4.2)</td>
<td>96.1 (95.5-96.6)</td>
<td>4.9 (4.7-5.1)</td>
<td>48/145 (33%)</td>
<td>68/135 (50%)</td>
<td>19/145 (13%) †‡</td>
<td>1.72 (1.61-1.84)††</td>
<td>94.5 (82.6-1064)</td>
</tr>
<tr>
<td>Human rhinovirus only</td>
<td>6</td>
<td>7.1 (1.7-4.2) ‡‡ (p=0.01)</td>
<td>96.3 (94.1-98.6)</td>
<td>4.7 (4.1-5.2)</td>
<td>0/6</td>
<td>0/6 (p=0.03)</td>
<td>0/6</td>
<td>1</td>
<td>64.4 (17.4-111.4)</td>
</tr>
<tr>
<td>Respiratory syncytial</td>
<td>13</td>
<td>5.8 (3.5-8.1) ‡‡ (p=0.04)</td>
<td>94.9 (91.4-98.4)</td>
<td>5.2 (4.7-5.8)</td>
<td>4/13 (31%)</td>
<td>7/12 (58%)</td>
<td>1/13 (8%)</td>
<td>1.62 (1.22-2.01)</td>
<td>123.0 (90.3-155.9)‡‡</td>
</tr>
</tbody>
</table>

---

1. **Table E2: Frequencies, use of supportive care and LOS by virus with high genomic load only**

2. *p=0.006, †p=0.021, **p=0.004 adjusted for age in logistic regression; ‡p=0.004, $p=0.042, ††p=0.004, ‡‡p=0.046, in robust linear regression adjusted for age (estimated men).

3. Unadjusted analyses: *p=0.005, †p=0.014, **p=0.001 in Pearson Chi-squared analyses. ‡p=0.001, $p=0.038, ††p=0.001, ‡‡p=0.12, in robust linear regression analyses.

4. In the analyses presented in this table, only a high viral genomic load is classified as positive, while moderate, low or no viral genomic load is classified as negative.
Figures

Figure E1 Cluster analyses

Number of patients at each semi-quantitative concentration. Unclustered level of PCR threshold cycle (delta Ct - x-axis on all graphs) values (top) and grouped into five clusters (bottom) for each virus independently. The highest genomic load clusters are marked with blue, the four lower load clusters with red.
Figure E2 Monoinfections per virus
Figure E3: The effect of inhaled racemic adrenaline compared to saline on the mean length of stay (estimated in robust linear regression analyses) in subgroups by viral diagnostics.