“High incidence of pulmonary bacterial co-infection in children with severe respiratory syncytial virus (RSV) bronchiolitis”

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Abstract

Introduction: RSV is the most common cause of viral lower respiratory tract infections (LRTI). Viral LRTI is a risk factor for bacterial superinfection with an escalating incidence with increasing severity of respiratory illness.

Objectives: 1) To determine the incidence of pulmonary bacterial co-infection in infants and children with severe RSV bronchiolitis, using paediatric intensive care unit (PICU) admission as a surrogate marker of severity.

2) To study the impact of the co-infection on morbidity and mortality.

Methods: Prospective microbiological analysis of lower airways secretions on all RSV positive bronchiolitis patients on admission to the PICU during three consecutive RSV seasons.

Results: 165 of the 181 children (median age 1.6 months, IQR 0.5 – 4.6) admitted to PICU with RSV bronchiolitis were enrolled, as an admission broncho-alveolar lavage (BAL) sample was not available in 16 patients (8.8%). 70 (42.4%) children had lower airway secretions positive for bacteria – 36 (21.8%) children were co-infected and another 34 (20.6%) had low bacterial growth/possible co-infection. All were mechanically ventilated (median 5.0 days, IQR 3.0 – 7.3). Those with bacterial co-infection required ventilatory support for longer than those with only RSV (p < 0.01). White cell count, neutrophil count and CRP did not differentiate between the groups. 45% (74/165) received antibiotics prior to intubation. Gender, co-morbidity, origin, prior antibiotics, time on preceding antibiotics, admission oxygen and ventilation index were not predictive of positive bacterial cultures. There were twelve deaths (6.6%), five were RSV-related (2.8%).

Conclusions: Up to 40% of patients with severe RSV bronchiolitis requiring PICU admission were infected with bacteria in their lower airways and were at increased risk for bacterial pneumonia.
Introduction

Respiratory syncytial virus (RSV) is the most important viral cause for lower respiratory infection in infants and young children throughout the world.[1] It is one of the commonest causes of respiratory tract infection leading to respiratory failure. It has been estimated that in each year 600,000 deaths occur worldwide that are directly or indirectly attributable to RSV.[1] Factors that increase susceptibility to the virus include chronological age less than 6 weeks, bronchopulmonary dysplasia, congenital heart disease, prematurity, and immunodeficiency.[2][3][4][5] Although the mortality rate for those hospitalized may be as low as 1 – 3%, the mortality rate increases in those with severe bronchiolitis requiring intensive care management.[2][3][4][5][6] In developed countries about 2% of infants and children admitted to hospital with RSV require assisted ventilation.[7] RSV bronchiolitis is a common cause for admission to a paediatric intensive care unit (PICU) in the winter season.[5][6][7].

The pharmacological management of RSV bronchiolitis, other than the use of supplementary oxygen, has long been debated.[8][9] In particular, many advocate against the routine use of antibiotics in bronchiolitis on account of a reported low incidence of concurrent or secondary bacterial infections in RSV patients.[10][11][12][13][14][15][16][17] However, these studies focused on extrapulmonary bacterial co-infection and included only limited numbers of children with severe respiratory compromise/failure.

Physiologically the lower airways are normally sterile. Nevertheless, the relationship of bacterial co-infection with viral respiratory disease has been recognised previously, with an escalating incidence with increasing severity of respiratory illness.[18] Three retrospective studies investigated the occurrence of bacterial co-infection in children with severe RSV infection requiring PICU admission and found the incidence of pulmonary bacterial co-infection to vary between 17.5% - 44%.[19][20][21]. In this study we prospectively investigated the incidence of pulmonary bacterial co-infection using established quantitative microbiology.[22] in patients with severe RSV bronchiolitis on admission to a tertiary PICU and evaluated the impact of the bacterial co-infection on morbidity and mortality.
Patients and methods

Setting: Children admitted to the PICU at the Royal Liverpool Children’s Hospital, a university-affiliated, multi-disciplinary, regional referral centre, were studied. The PICU is a 20-bedded facility with an annual admission rate of over 1000 children. The overall mortality rate is 4.5%, with a predicted mortality of 6.25% using Paediatric Index of Mortality (PIM)[23] and a standardized mortality rate of 0.72.

Objectives:
1) To determine the incidence of pulmonary bacterial co-infection in patients requiring PICU for severe RSV bronchiolitis.
2) To study the impact of the co-infection on morbidity, including length of ventilation and inflammation, and mortality.

Inclusion criteria:
Children with RSV bronchiolitis, confirmed on RSV antigen testing and/or culture, requiring PICU and on whom lower airway secretions were obtained on admission.

Exclusion criteria:
Nosocomial cases of RSV infection in the PICU.

Study design: The data was collected prospectively during 3 consecutive RSV seasons (winter seasons) between 2002 and 2005 in the cohort of RSV-positive children admitted to PICU. The study was approved by the Institutional Ethics Review Board.

Respiratory support: Intubation was performed by our PICU retrieval team at the referring hospital, in our accident & emergency (A&E) department, or in one of the hospital wards prior to PICU admission. Alternatively, the anaesthetic team of the referring hospital intubated some of the patients preceding the arrival of our PICU retrieval team. It is policy that all children who require intensive care and ventilatory support are moved to the regional PICU. The timing of extubation was judged clinically and not influenced by bronchoalveolar lavage (BAL) results.

Microbiologic sampling: Diagnostic samples of nasopharyngeal aspirates (for RSV detection) and lower airway secretions (for bacterial culture) through endotracheal tube using sterile precautions[24] were taken on admission and processed immediately in the laboratory. Prior to routine bronchial toilet a sterile suction catheter was passed down the endotracheal tube. Two 1ml/kg aliquots of sterile 0.9% saline were instilled through the suction catheter, immediately followed by aspiration with constant pressure into a mucus trap. Samples were collected by specialist respiratory physiotherapists or PICU staff members. BAL was performed immediately after endotracheal intubation in children intubated in our hospital and on arrival in the PICU - generally within three hours of endotracheal intubation for those retrieved from other hospitals. All children within the region are only ventilated in the regional PICU, so are rapidly transferred to the PICU.
Surveillance samples of throat and rectum were obtained on admission and then twice weekly, in keeping with the routine surveillance practice in our unit.
Laboratory procedures: Viral - Nasopharyngeal aspirates were tested by the Directigen™ RSV test [Becton Dickinson microbiology systems, Maryland, USA]. This is an in vitro enzyme immunoassay [ELISA] membrane test for the rapid and qualitative detection of RSV antigen directly from nasopharyngeal specimens. All samples negative for RSV using the ELISA membrane test were cultured using standard virological techniques at the Health Protection Agency. Bacterial/Yeast - Diagnostic or clinical samples were processed immediately in a qualitative and semi-quantitative way using standard microbiological methods. For all types of samples, macroscopically distinct colonies were isolated in pure culture. Standard methods for identification, typing, and sensitivity patterns were used for all micro-organisms.[25]

Antibiotic policies
Patients with signs of infection received intravenous cefotaxime (150 mg/kg/day 4 times daily for up to 7 days) as first line therapy for 48 hours whilst awaiting culture results. Clinical status on presentation governed whether supplementary intravenous cover with an aminoglycoside, gentamicin (7.5 mg/kg/day 3 times daily for up to 7 days) was added. Antibiotics were rationalized once culture and sensitivity results became available.

Definitions:
Bacteria positive - The presence of micro-organisms in the lower airways which is normally sterile.
Co-infection - Infection is a microbiologically proven, clinical diagnosis of inflammation, local and/or generalized. In this study clinical signs were unreliable as all patients had bronchiolitis, so microbiological definitions were utilized. Bacterial co-infection required bacteria colony counts ≥10^5 CFU/ml of diagnostic sample for each single species obtained from lower airway secretions and, on a semi-quantitative scale of + = few (<50 X 10^6/L), ++ = moderate (>100 X 10^6/L) and +++ = many leucocytes (>1000 X 10^6/L), the presence of at least a moderate [++] number of leucocytes.[26][27][28]
Low bacterial growth - Diagnostic samples from lower airway secretions which yielded <10^5 CFU/ml of diagnostic sample and only a few [+ ] leucocytes.

Chest Xray appearance was not utilized to diagnose bacterial co-infection as chest Xray changes are not pathognomonic of secondary bacterial or viral infections.[18][29]

Analytic Methods:
Data were collected prospectively. Prediction of mortality using paediatric index of mortality (PIM) was obtained on the patient’s first contact with the PICU team.[23] Results were expressed as a percentage of the total study population; median and inter-quartile ranges (IQR) were used to describe the demographic distributions. Continuous data was analyzed using the Wilcoxon-Mann-Whitney (W-M-W) test. Categorical data was analyzed using Fisher’s exact or McNemar’s test. Correlation was assessed using Spearman’s rank test (two-tailed). Multivariate analysis was performed using linear and logistic regression analysis.
Statistical calculations were performed with the Statistical Program for Social Science release 11.0.0 (SPSS 11, Chicago, IL). A p value < 0.05 was considered statistically significant.

**Results**

A total of 181 children were admitted to the PICU with RSV positive bronchiolitis during the three consecutive RSV seasons (2002 – 2005). The indication for PICU admission for these children was ventilatory/respiratory support (respiratory failure (172) and/or life-threatening apnoeas (9)). All patients were mechanically ventilated for a median of 5.0 days [IQR 3.0 – 7.3]. Median age was 1.6 months [IQR 0.5 – 4.6]. 103 were boys and 78 girls. 165 children were enrolled in the study – an admission bronco-alveolar lavage (BAL) sample was not available in 16 patients (8.8%).

Table 1 shows the demographics, inflammatory marker values, antibiotic history and mortality of the RSV-positive critically ill children in the subgroups: RSV only, bacterial co-infection, low bacterial growth and bacteria positive (co-infection + low bacterial growth). Admission white cell count, neutrophil count and CRP did not differentiate between the groups, and neither did these indices during PICU days 1 – 5.

Although all patients were admitted primarily for respiratory disease, 43% (71/165) of them had other co-morbidities – congenital heart disease 37, chronic lung disease 8, immunodeficiencies 4, abnormality of large airways 5, congenital heart disease and abnormality of large airways 8, congenital heart disease and chronic lung disease 4, neuromuscular disease 7. Co-morbidity did not increase the risk of positive bacterial cultures (odds ratio = 0.77, 95% CI 0.55 to 1.09).

Overall 45% (74/165) received antibiotics prior to PICU admission (i.e. started by the referring hospital or ward), most often cefotaxime or ceftiraxone. The breakdown between the subgroups is shown in Table 1. Receipt of antibiotics prior to PICU admission did not affect PIM (W-M-W test p = 0.6) and LOV (W-M-W test p = 0.2). All patients, bar eight, were continued or commenced on antibiotics in the PICU – usually cefotaxime. Antibiotics were continued for a median of 5 days [IQR: 3 – 6 days]. The empiric use of antibiotics was at the discretion of the attending consultant.

Gender, age groups, PIM, co-morbidity, receipt of prior antibiotics, time on antibiotics prior to intubation, admission oxygen and ventilation index were not predictive of positive bacterial cultures by uni- or multivariate analysis (all p-values > 0.16).

The organisms that were isolated from lower airway secretions obtained on admission are shown in Table 2. All those with positive endotracheal bacteriology, had the same organisms isolated on admission surveillance swabs. Community organisms accounted for 83% (81/98) of the bacteria cultured.

There were twelve deaths (6.6%), of which 5 appeared to be RSV-related (2.8%) as the patients were still RSV positive when they died. Two were
oncology patients (leukaemias on chemotherapy) that died from RSV pneumonitis on day 1 and 16 respectively. Neither had proven bacterial co-infection and both received broad spectrum empiric antibiotic therapy. Other associated causes included singles cases of: hypoplastic right heart coupled with cystic fibrosis (on day 8), *B. pertussis* co-infection with hypoxaemic respiratory failure requiring extracorporeal membrane oxygenation (ECMO) (on day 26), and a child with a congenital myopathy (on day 8). The remaining 7 deaths occurred 6 – 31 days after admission subsequent to the RSV cultures becoming negative. Causes of these RSV ‘un-related’ deaths included: complex congenital heart disease 3, multiple congenital anomalies 2, congenital myopathy 1, anoxic brain injury 1. Positive bacterial cultures did not predict death (odds ratio = 1.3, 95% CI 0.57 to 2.95), but co-morbidity did (odds ratio = 0.51, 95% CI 0.37 to 0.7).
Table 1: Patient characteristics according to culture result – median [interquartile range].

<table>
<thead>
<tr>
<th></th>
<th>RSV only</th>
<th>Bacterial co-infection (&gt; $10^5$ CFU/ml)</th>
<th>Low bacterial growth (&lt; $10^5$ CFU/ml)</th>
<th>Bacteria positive (Co-infection + low bacterial growth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (total 165) and percentage of total number</td>
<td>95</td>
<td>36</td>
<td>34</td>
<td>70</td>
</tr>
<tr>
<td>Age (months)</td>
<td>1.4 [0.4 – 3.9]</td>
<td>1.3 [0.7 – 2.5]</td>
<td>3.5 [1.2 – 10]</td>
<td>1.8 [0.9 – 4.6]</td>
</tr>
<tr>
<td>Paediatric Index of Mortality (PIM)</td>
<td>0.08 [0.03 – 0.12]</td>
<td>0.09 [0.04 – 0.14]</td>
<td>0.08 [0.06 – 0.12]</td>
<td>0.08 [0.05 – 0.13]</td>
</tr>
<tr>
<td>Antibiotics prior to PICU admission</td>
<td>48%</td>
<td>36%</td>
<td>44%</td>
<td>40%</td>
</tr>
<tr>
<td>Mortality: (RSV-related deaths)</td>
<td>8 (3)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Percentage with co-morbidities§</td>
<td>40%</td>
<td>61%</td>
<td>41%</td>
<td>51%</td>
</tr>
</tbody>
</table>

CFU/ml = colony forming units of a single bacterial species per ml of diagnostic sample
Retrieved = patients retrieved from other hospitals
Intra-hospital = patients admitted from wards with our hospital
A&E = patients admitted directly from the Accident & Emergency department

* RSV-only vs. Bacterial co-infection     † RSV-only vs. Low bacteria growth
‡ RSV-only vs. All those positive for bacteria (bacterial co-infection + low bacteria growth)
Wilcoxon-Mann-Whitney test used, except for prior antibiotics and co-morbidities – McNemar’s test.

§ Co-morbidities = congenital heart, chronic lung disease, abnormality of large airways, immunodeficiencies, neuromuscular disease

OI = mean airways pressure (MAP) x FiO₂/PaO₂
VI = respiratory rate x PaCO₂ x peak inspiratory pressure / 1000
Table 2: The 98 bacterial isolates obtained on admission to PICU from the lower airway in 70 children with severe RSV bronchiolitis:

<table>
<thead>
<tr>
<th></th>
<th>Co-infection (&gt; 10^5 CFU/ml)</th>
<th>Low bacterial growth (&lt; 10^5 CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Community organisms</strong>&lt;sup&gt;28,30&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Abnormal organisms</strong>&lt;sup&gt;28,30&lt;/sup&gt; ¶</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><em>B. pertussis</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>E. cloacae and C. freundii</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>MRSA</em></td>
<td></td>
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</tr>
</tbody>
</table>

23 patients had multiple organisms (18 had two, 5 had three bacteria) – Community organisms were involved in 91% of these cases, compared to 77% of single isolates. (Fisher’s exact p = 0.2) ¶ 67% (10/15) had chronic illnesses
Discussion

This observational study over 3 consecutive RSV seasons evaluating bacterial pulmonary co-infection found that 42% of children admitted for severe RSV infection harboured bacterial pathogens in their lower airways. These critically ill children run a serious risk of developing bacterial pneumonia [18][31].

BAL samples were collected very soon after intubation so significant growth densities of bacteria reflect pathogens in the normally sterile lower airways. The high number of colony forming units makes it highly unlikely that the microorganisms isolated were ‘pushed down’ the trachea on intubation. We acknowledge that the number of leukocytes in lower airways secretions will also be influenced by RSV infection and therefore relied on bacterial growth. The microbiological criteria were strict and avoided potentially confounding clinical factors. This microbiological approach is supported by recent literature concerning ventilator-associated pneumonia (VAP).[32][33] On the other hand, it must be appreciated that our study group was very different from this VAP group, as they had ‘virgin’, iatrogenically uncontaminated lower airways. Certainly the group defined as co-infection had substantial bacterial growth densities far too soon after intubation to have been oropharyngeal flora transported there by the endotracheal tube. If anything, the strict microbiological criteria probably underestimated the number co-infected by categorizing many of them as low bacterial growth. We accept that differentiating the groups into co-infected and low bacterial growth may be somewhat artificial as the lower respiratory tract should be free from bacteria.

The term “co-infection” was used, as, at the time of PICU admission, these infections could either be secondary or concurrent. It would not be easy to detect the ‘chicken’ from the ‘egg’ as far as which was primary, the RSV or the bacteria, although a viral infection destroying cilia is in general required for a bacterial co-infection.[18] The true co-infection rate is likely to be higher than the 22% rate detected, as 45% of the cases received antibiotics prior to PICU admission. These antecedent antibiotics may have converted some of the “co-infection” patients into the “low bacterial growth” group, or even prevented bacterial growth altogether.

Previous studies have looked at bloodstream, otitis media or urinary tract infections in children with bronchiolitis, very few of whom had severe RSV bronchiolitis requiring intensive care.[10][11][12][13][14][15][16][17] These studies generally found a very low incidence of secondary serious bacterial infection (1.2%)[14] or bacteraemia (0.6%)[16] in their hospitalized RSV patients. Because these studies did not specifically concentrate on those with severe bronchiolitis, it is difficult to extrapolate their results to this population. Duttweiler et al retrospectively studied 127 infants admitted to intensive care for RSV bronchiolitis and found that 25 (44%) of the 57 ventilated and endotracheally sampled infants had “concomitant bacteria pneumonia”. [19] Similarly, the retrospective study of Kneyber et al (82 PICU admissions with 65 or 79% ventilated) found that 9 (33%) of the 24 children on whom admission endotracheal aspirates were performed had a positive bacterial culture.[20] Randolph et al retrospectively examined 165 previously healthy infants admitted to the intensive care unit over a 12-year period with laboratory-
confirmed RSV infection (63 or 38% required mechanical ventilation). They found that 17.5% – 38% of the 63 intubated infants had “probable” or “possible” bacterial pneumonia.[21] The incidence of bacterial pulmonary infection in these retrospective PICU reports is in keeping with that of this prospective study in which all bronchiolitic admissions were included.

51% of the patients with bacteria in their airways and 40% of the RSV-only children had co-morbidities - congenital heart disease, chronic lung disease, large airway abnormality, immunodeficiency, neuromuscular disease. This is in keeping with well-recognised risk factors associated with more severe RSV disease.[3][4][6][16][34] Co-morbidities did not account for differences in length of ventilation amongst the study groups, but did contribute towards mortality. The high percentage with co-morbidities is most probably also influenced by our centre being the regional paediatric cardiac referral centre, thereby resulting in children with congenital heart disease and bronchiolitis being more likely to be referred to our PICU for intensive care management.

There were fewer deaths in the bacteria-positive group than in those with RSV only. However, when adjusted for those children who had recovered from their RSV infection only to demise later from RSV-unrelated causes, both groups had similar mortalities (2.9% vs. 3.2%). Paediatric Index of Mortality (PIM) is a point of first contact score that is used to assess the risk of death while in the PICU.[23] The PIM scores for all the groups were similar, suggesting that all groups had matching severity of illness on admission to the PICU. Yet those with positive bacterial cultures required ventilatory support for longer than those with RSV only. Kneyber et al found likewise.[20] Although length of ventilation was significantly different between the groups, other respiratory support and inflammation indices did not differentiate between them (Table 1). Perhaps the general inflammatory response once triggered by RSV is not so refined as to be further enhanced by concomitant bacterial infection. Others have also found inflammatory markers unhelpful in differentiating bacterial infection in this group of patients.[20][35][36] Unfortunately, we were unable to find any early clinical measurements which would identify those RSV patients with bacterial co-infection.

Receipt of and length of time on prior antibiotics did not predispose to bacterial co-infection. Moreover, many of the RSV children had had only a day or less (often a single dose close to intubation) of antibiotics. The fact that nearly all the RSV positive children received antibiotics in our PICU limited any interpretation on the impact of antibiotics on their outcome. All those patients with positive bacteriology in their endotracheal secretions had the same organisms isolated on admission surveillance swabs, indicating primary endogenous infection.[37] This reinforces the pathogenesis of lower airways infections in that potential pathogens are carried first in the nasopharynx and then there is migration down the trachea into the lower airways.[37][38] The organisms isolated on admission were generally normal community organisms because the majority of the patients were in good health prior to RSV infection and PICU admission.[27][29] In contrast, *Pseudomonas aeruginosa* was the most common of the abnormal bacteria (Table 2). All these patients were carriers of abnormal organisms in their throats, and in most the common
denominator for their abnormal carriage was chronic illness.[37][39] Interestingly, *Streptococcus pneumoniae* was isolated from relatively few patients. This could be the result of prior antibiotic use.[40]

Although the majority of LRTI in children are viral in aetiology, mixed viral-bacterial infections are seen in up to a quarter of hospitalized children.[41][42] Additionally there is the risk of developing bacterial superinfection with viral LTRI.[18] These issues have played a role in the World Health Organization’s recommendations for the treatment of community-acquired pneumonia to include empirical antibiotics.[41][43] Concerns that using antibiotics (in our case cefotaxime) pre-emptively in this group of critically ill children would breed antibiotic resistance have been shown to be unfounded in a four-year study.[44] The assessment of the influence of antibiotics on children with severe bronchiolitis would require a prospective randomized control trial.

Conclusions:
Up to 40% of patients admitted with severe RSV bronchiolitis were infected with bacteria in their lower airways. Co-morbidity, namely congenital heart disease, chronic lung disease, large airway abnormality, immunodeficiency, neuromuscular disease, predispose to more severe RSV disease.

The authors have no financial or ethical conflicts of interest, or any other competing interests, regarding the contents of this manuscript.
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