Mechanical Ventilation Drives Inflammation in Severe Viral Bronchiolitis

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Abstract

Introduction: Respiratory insufficiency due to severe respiratory syncytial virus (RSV) infection is the most frequent cause of paediatric intensive care unit admission in infants during the winter season. Previous studies have shown increased levels of inflammatory mediators in airways of mechanically ventilated children compared to spontaneous breathing children with viral bronchiolitis. In this prospective observational multi-center study we aimed to investigate whether this increase was related to disease severity or caused by mechanical ventilation.

Materials and Methods: Nasopharyngeal aspirates were collected <1 hour before intubation and 24 hours later in RSV bronchiolitis patients with respiratory failure (n = 18) and non-ventilated RSV bronchiolitis controls (n = 18). Concentrations of the following cytokines were measured: interleukin (IL)-1α, IL-1β, IL-6, monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α.

Results: Baseline cytokine levels were comparable between ventilated and non-ventilated infants. After 24 hours of mechanical ventilation mean cytokine levels, except for MIP-1α, were elevated compared to non-ventilated infected controls: IL-1α (159 versus 4 pg/ml, p < 0.01), IL-1β (1068 versus 99 pg/ml, p < 0.01), IL-6 (2343 versus 958 pg/ml, p < 0.05) and MCP-1 (174 versus 26 pg/ml, p < 0.05).

Conclusions: Using pre- and post-intubation observations, this study suggests that endotracheal intubation and subsequent mechanical ventilation cause a robust pulmonary inflammation in infants with RSV bronchiolitis.

Introduction

Respiratory syncytial virus (RSV) is the most common viral cause of seasonal acute respiratory tract illness in infants worldwide. The clinical manifestations range from mild upper respiratory tract symptoms (cough, coryza, rhinorhoea and conjunctivitis), to severe lower respiratory tract infection (LRTI) and even life-threatening respiratory insufficiency requiring mechanical ventilation. Treatment for RSV LRTI is largely supportive and no effective vaccine is currently available [1]. More than 50% of all infants are infected with RSV during the first year of life and at age 2 almost all children have been infected [2]. About 1% to 2% of all children will need hospitalization and about 10% of these hospitalized children, approximately 0.1% of all children, will require mechanical ventilation for a severe RSV LRTI during the first year of life [3]. Accordingly, RSV LRTI is the most frequent cause of non-elective paediatric intensive care unit (PICU) admission for mechanical ventilatory support in infants during the winter season [4].

RSV infection is associated with the production and release of large amounts of proinflammatory cytokines and chemokines [5,6]. Several studies have demonstrated a correlation between disease severity with both local immune response [7–10] and viral load [11–14]. When comparing ventilated with non-ventilated infected infants, an association between the inflammatory response and disease severity was also found [14–17]. In these studies however, all samples from ventilated infected infants were collected after initiation of mechanical ventilation. Subsequently, observed cytokine concentrations might not only reflect virus-induced pulmonary inflammation but also the immunological response to mechanical ventilation known as ventilator induced lung injury [18].

We sought to investigate to what extent increased local inflammation in mechanically ventilated children with RSV bronchiolitis resulted from disease severity or endotracheal intubation and initial mechanical ventilation. We hypothesized that differences in cytokine levels between mechanically ventilated and non-ventilated RSV infected infants were absent at time of
intubation but developed during the early phase of mechanical ventilation. To test this hypothesis we studied local cytokine levels prior to endotracheal intubation and again 24 hours later.

**Materials and Methods**

The study was approved by the regional Medical Ethical Committee South West Holland and the Ethics Review Committee of the University Medical Center Utrecht and conducted according to the principles expressed in the Declaration of Helsinki. All parents provided written, informed consent.

**Selection of patients**

Infants less than 13 months old with a proven RSV infection and respiratory insufficiency requiring mechanical ventilation were enrolled in the Wilhelmina’s Children’s Hospital, Utrecht, the Netherlands during 2 winter epidemics (2009–2011). The decision to intubate and start mechanical ventilation was at the discretion of the attending physician. During the same period, hospitalized children less than 13 months with proven RSV LRTI without the need for mechanical ventilation were enrolled as non-ventilated controls in two hospitals in the Netherlands (Wilhelmina’s Children’s Hospital, Utrecht and Juliana Children’s Hospital/ Haga Teaching Hospital, The Hague). Infants with underlying chronic lung disease, cyanotic congenital heart disease, Down’s syndrome and/or prematurity (gestational age < 37 weeks) were excluded.

**Collection of materials**

For this study we measured cytokine levels in nasopharyngeal aspirates (NPA). A strong correlation exists between cytokine concentrations determined in upper and lower airways [9,15,17,19] In order to collect material before the onset of mechanical ventilation as well as to optimize the overall sample collection rate, undiluted NPAs were collected shortly (< 1 hour) before intubation. A second NPA was collected 24 hours after intubation. In non-ventilated patients, a NPA sample was taken on admission and 24 hours later. Aspirates were placed on ice immediately and stored at –80°C for later cytokine analysis.

**Measurements**

Interleukin (IL)-1α, IL-1β, IL-6, monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α were measured in NPAs by enzyme-linked immunosorbent assay according to manufacturer’s instructions (R&D Systems, United Kingdom). The lower detection limit for IL-1α was 8 pg/ml; for IL-1β 70 pg/ml, for IL-6 32 pg/ml, for MCP-1 32 pg/ml and for MIP-1α 80 pg/ml. When cytokines were not detectable, the minimum detectable level divided by 2 was used in the calculations. RSV infection was diagnosed by PCR as described previously [14]. RSV concentrations were analysed using real-time PCR. Low cycle time (CT) values indicate high RSV concentrations, while high CT values (with a maximum of 40 cycles) represent low viral loads.

**Statistical analysis**

All data are expressed as mean (±SD). To detect differences between groups a paired t-Test was used and a p-value of less than 0.05 was considered statistically significant. Correlation between viral load and cytokine concentration was analysed with Spearman’s rank correlation coefficient. Statistical analyses were carried out using GraphPad PRISM 5 (La Jolla, United States of America).

**Table 1. Patient characteristics of spontaneous breathing and mechanically ventilated RSV-infected infants.**

|                        | Spontaneous Breathing | Mechanically Ventilated | p-value |
|------------------------|------------------------|-------------------------|---------|
| Nr patients included   | 18                     | 18                      | NS      |
| Male patients, nr (%)  | 11 (61)                | 11 (61)                 | NS      |
| Age in days, mean (SD) | 95 (57)                | 72 (90)                 | 0.04    |
| Weight in kg, mean (SD)| 6.1 (5)                | 4.7 (1.4)               | 0.02    |
| Days ill at t = 0, mean (SD) | 2.8 (3) | 3.7 (2) | 0.11 |
| Viral co-infections, nr (%) | -           | 3 (17)                   |         |
| Bacterial pneumonia, nr (%) | -              | 6 (33)                   |         |

**T = 0 hours**

|                        |                        |                        |
|------------------------|------------------------|------------------------|
| Blood gas analysis, nr (%) | 5 (28)       | 12 (67)                |
| pH/PaCO2/BE/Bic, mean (SD) | 7.39(0.04)/53.7(12.3)/28.6(3) | 7.19(0.08)/72.4(22.1)/25.9(8) |

**T = 24 hours**

|                        |                        |                        |
|------------------------|------------------------|------------------------|
| Vt in ml/ kg, mean (SD)|                        | 7.6 (2)                |
| Peak ventilator pressure in cm H2O, mean (SD)- | 27.5 (5) |
| Ventilator FiO2, mean (SD) | -                      | 0.37 (0.05) |
| Transcutaneous saturation, mean (SD) | 98 (1) | 98 (2) |
| Blood gas analysis, nr (%) | 1 (6)          | 18 (100)               |
| pH/PaCO2/BaO2/Bic, mean (SD) | 7.32(0.0)/65.0(0)/8.0(0)/33.8(0) | 7.38(0.05)/45.3(7)/2.2(3)/27.2(3) |

Nr: number of patients; kg: kilograms; T = 0 for ventilated patients is < 1 hour before intubation, for non-ventilated patients on admission; T = 24 is 24 hours after the first sample in both groups; NS: not significant; PaCO2: arterial carbon dioxide tension; BE: base excess; Bic: bicarbonate; FiO2: fraction of inspired oxygen; Vt: Tidal volume. 

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Results

Patient Characteristics

Characteristics of patients and controls are shown in table 1. During the study period 37 RSV-infected patients were referred to our PICU for ventilatory support resulting in inclusion of 18 patients (figure 1). At time of sampling, six patients in this study group had positive bacterial cultures from tracheal aspirates (Moraxella catarrhalis n=2, Haemophilus influenza n=4, Streptococcus pneumoniae n=2, Staphylococcus aureus n=3, Streptococcus pyogenes n=1) whereas 3 others had a viral co-infection (Rhinovirus n=3, Adenovirus n=1). None of the patients received ribavarin or systemic steroids. All patients survived.

Viral loads

Although nasopharyngeal viral loads taken on t = 0 in ventilated infants were higher than loads measured in the spontaneous breathing group (CT 20.6 vs. 22.9), this difference did not reach statistical significance (p = 0.06). There was no correlation found between viral loads and cytokine levels.

Discussion

This is the first study investigating cytokine levels before and after endotracheal intubation for RSV bronchiolitis. We studied to what extent intubation and subsequent mechanical ventilation in infants with RSV bronchiolitis could have impacted on pulmonary inflammation in comparison with infants with RSV bronchiolitis.
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who were not mechanically ventilated. We found that the need for mechanical ventilation in RSV-infected infants was not associated with enhanced virus-induced pulmonary inflammation at baseline. Endotracheal intubation and subsequent mechanical ventilation induced additional inflammation expressed by increased levels of NPA cytokine concentrations after 24 hours.

The association between immunological response and disease severity in spontaneous breathing versus mechanically ventilated RSV infected children has been previously studied by our group and others [14–16]. In these studies however cytokine analysis was performed on samples collected while patients were already ventilated. Therefore, observed cytokine concentrations might not only reflect viral induced pulmonary inflammation but also the immunological response to intubation and mechanical ventilation which complicates interpretation of these results. However, we now show that virus induced pulmonary inflammation in infants with mild RSV disease (spontaneous breathing group) was similar to what was observed in infants with severe RSV disease (ventilated group) at time of intubation. Hence, the need for mechanical ventilation in previously healthy infants was not explained by enhanced viral induced pulmonary inflammation or viral load. Most likely, other patient-related factors, such as age and/or weight [20] and genetic susceptibility [16] played an important role in the development of life-threatening RSV bronchiolitis.

The increase in cytokine levels in infants with respiratory failure due to RSV bronchiolitis was attributable to intubation and mechanical ventilation, despite the use of mean tidal volumes of 7.6 ml/kg, reflecting a so-called lung protective ventilatory strategy [21–24]. Furthermore, disease duration at presentation did not differ between the 2 groups but might be attributable to the small number of patients studied. Subsequently, we compared the 2 groups at similar time points: baseline sample of ventilated children taken after 3.8 days of disease. No differences were found, except for IL-1α (data not shown). In addition, virus induced inflammation did not increase during the first 24 hours in spontaneous breathing controls. This strengthens the observation that enhanced inflammation is indeed attributable to ventilator induced inflammation. These findings corroborate experimental studies demonstrating that mechanical ventilation augments pulmonary inflammation during viral infection [25,26]. As we have previously shown, the current study provides further support that inflammation in ventilated children is attributable to a ventilator induced molecular stress response superimposed on virus-induced innate immunity activation with little interaction [26].

The current study adds to our knowledge of the inflammatory consequences of endotracheal intubation and initial ventilation: a robust inflammatory response is superimposed on top of existing viral induced inflammation. Furthermore it underscores the need to adhere to lung protective ventilation strategies in patients with severe respiratory infections. More importantly, one could argue that given the observed additional inflammatory hit resulting from invasive mechanical ventilation, non-invasive ventilatory strategies may be considered for infants with life-threatening RSV bronchiolitis to prevent further airway inflammation.

This study has several strengths. Firstly, even in healthy children mechanical ventilation elicits an inflammatory response within hours [27]. Therefore, a baseline sample just prior to intubation, as was collected in our study, was essential. Secondly, the observed enhanced inflammatory response during mechanical ventilation was observed while using a lung protective mode of mechanical ventilation. On the other hand, we have to consider the following limitations. Firstly, mechanically ventilated infants were significantly younger than their spontaneous breathing controls. However, we have previously shown that neonatal innate immune responses in whole blood are biased against the production of pro-inflammatory cytokines at birth and this production gradually increases during the first month of life [28]. No studies have investigated age dependent cytokinesis production in nasal specimens. However, in light of fetomaternal tolerance, it seems likely that neonatal production of proinflammatory cytokines is generally impaired [29]. Therefore, the pro-inflammatory response seen in our ventilated group cannot be explained by their younger age. Secondly, a healthy age-matched control group requiring invasive mechanical ventilation for at least 24 hours was lacking for practical reasons. This group however could have expanded our knowledge on the immunological effects of invasive mechanical ventilation in normal lungs and on the effects of stress, pain and sedation in intubated infants. Thirdly, a RSV-infected control group matched for age and disease severity not exposed to invasive mechanical ventilation was desirable. Unfortunately, this was not feasible during the study period as non-invasive mechanical ventilation was not yet available in our paediatric intensive care unit. Finally, we did not distinguish viral co-infection because these were uncommon (n = 3). Bacterial pulmonary co-infections are common in severe RSV-infections requiring PICU admission [30] and might also influence pulmonary inflammation. Six of our ventilated patients (33%) were pragmatically diagnosed with concurrent bacterial pneumonia. Cytokine
concentrations of these 6 patients however did not differ from ventilated patients without concurrent bacterial pneumonia (data not shown).

In summary, we provide the first evidence that the need for mechanical ventilation in RSV-infected infants is not preceded by enhanced viral induced pulmonary inflammation. We showed that invasive mechanical ventilation is likely to aggravate pulmonary inflammation, warranting further development of lung-protective non-invasive ventilatory support for this group of patients.

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Author Contributions

Conceived and designed the experiments: MPH AJV LJB. Performed the experiments: MPH MB LJB. Analyzed the data: MPH AJV MB FB NJJ LJB. Contributed reagents/materials/analysis tools: MPH AJV MB FB NJJ LJB. Wrote the paper: MPH AJV MB FB NJJ LJB.