Surfactant Deficiency in Infants with Severe Acute Viral Bronchiolitis

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Objectives To evaluate surfactant content and function through the lamellar body count (LBC) and stable microbubble test (SMT) in mechanically ventilated infants with severe acute viral bronchiolitis.

Study design Controlled cross-sectional study of 32 infants receiving mechanical ventilation: 16 with a diagnosis of acute viral bronchiolitis and 16 with normal lungs. Tracheal fluid was collected and LBC was performed in an automated cell counter. Samples were kept frozen and thawed for testing. At the time of analysis, samples were diluted in a dithiothreitol solution, vortexed for 10 seconds, and aspirated by the cell counter. SMT was performed using the Pattle technique.

Results In the bronchiolitis group, the median (IQR) LBC was significantly lower than in the control group: 130 000 (61 250-362 250) vs 518 000 (180 250-896 000) lamellar bodies/μL; $P = .003$. Median (IQR) SMT values were also significantly lower in the bronchiolitis group: 10 (2-13) vs 400 (261-615) microbubbles/mm^2; $P < .001$.

Conclusions Infants with acute viral bronchiolitis have reduced surfactant content and function. We speculate that these simple tests may be useful to identify infants with bronchiolitis who would benefit from surfactant replacement therapy. *(J Pediatr 2014; ■: ■-■).*

Acute viral bronchiolitis is the most common lower respiratory tract infection of infants and children, and a common cause of hospitalization among children ≤3 years of age. It is mainly caused by the respiratory syncytial virus, which accounts for 50%-80% of cases, but other viral etiologies occur. The condition is seasonal, and causes inflammation and obstruction of the lower airways.[1-4]

It is speculated that bronchiolitis is associated with surfactant deficiency. Studies have reported changes in surfactant proteins and in surface tension measured with the pulsating bubble surfactometer or by the click test in tracheal aspirates from children with bronchiolitis.[5-7] In studies that tested the administration of exogenous surfactant, the predominant effects were a significant improvement in oxygenation, duration of mechanical ventilation, and length of pediatric intensive care unit (PICU) stay.[8-10]

Assessment of surfactant production and function in neonates has been performed by means of the lamellar body count (LBC) and stable microbubble test (SMT), which have yielded good accuracy for the diagnosis of respiratory distress syndrome (RDS).[11-13]

The objective of the present study was to assess surfactant content and function by means of the LBC and SMT tests in mechanically ventilated infants with severe acute viral bronchiolitis.

Methods

This study was conducted in the PICU of Hospital São Lucas, the teaching hospital of Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil, from June 2011 through March 2013. The study was approved by the institutional Research Ethics Committee and informed consent for participation was obtained from 1 of the parents or legal guardians of each patient.

The study sample included infants (age ≤12 months) admitted to the PICU with acute viral bronchiolitis receiving mechanical ventilation (bronchiolitis group). Diagnosis was established by the attending physician on the basis of the following clinical findings: first episode of acute-onset expiratory wheezing and signs of viral respiratory illness, such as nasal discharge, cough, and fever, accompanied by breathing difficulty.[14] The control group consisted of age-matched patients (age ≤12 months) who were on mechanical ventilation for postoperative management or other clinical conditions, regardless of whether they were admitted to the PICU, and who did not have any acute or chronic cardiopulmonary conditions. The purpose of this control

DTT	Dithiothreitol
LB	Lamellar body
LBC	Lamellar body count
PICU	Pediatric intensive care unit
RDS	Respiratory distress syndrome
SMB	Stable microbubble
SMT	Stable microbubble test

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group was to establish a normal reference range for lamellar body (LB) and stable microbubble (SMB) counts.

Data on demographics, ventilation settings at the time of tracheal aspirate collection, arterial blood gases, and markers of severity and clinical course—such as the pediatric index of mortality, PaO_2/fraction of inspired oxygen ratio, highest peak inspiratory pressure, duration of vasoactive drug use, and length of stay—were collected from patients in the bronchiolitis group. Furthermore, the most recent imaging studies of the lungs before tracheal aspirate collection were examined by a radiologist with particular experience in pediatric imaging. Radioimmunoassays were performed for viruses in tracheal aspirate specimens per routine PICU protocols. In both the bronchiolitis and the control groups, all treatment-related decisions were entirely at the discretion of the providers responsible for patient care.

Tracheal aspirate samples were obtained by a staff physical therapist per routine institutional protocols.[13] In short, mechanical respiration was briefly interrupted and a 0.5-mL aliquot of saline solution was instilled via the endotracheal tube. The patient was then reconnected to the ventilator. After a few respiratory cycles, the ventilator was disconnected again and a catheter was gently advanced through the endotracheal tube until resistance was felt. The tube was suctioned as the probe was gently withdrawn, and the resulting tracheal aspirate was collected into a closed suction system.

Samples were frozen and kept at −20°C for 48-96 hours. After thawing at room temperature, the SMT was performed by means of the adapted technique by Pattle et al.[13,15] All counting procedures were performed by an experienced examiner who was blinded to sample provenance. LBC was performed in the hematology laboratory of the Hospital São Lucas, the teaching hospital of Pontifícia Universidade Católica do Rio Grande do Sul.

To measure SMT, an aliquot of 40 μL of tracheal aspirate was suctioned into a Pasteur pipette (BRAND GMBH + CO KG, Wertheimer, Germany) with an 11.5 cm stem and 1 mm diameter and placed on a count chamber (Neubauer Improved Bright-Line, Optik Labor, Germany) without the slide cover. With the pipette held vertically, its tip almost touching the counting chamber, the aliquot was suctioned in and quickly expelled out 20 times. It was then expelled over the counting chamber, which was immediately inverted and placed under a binocular microscope, forming a hanging drop. After 4 minutes, the count area was examined with a magnification of 10 ×10, and the microbubbles—bubbles smaller than 15 μm—were counted. Five of the 25 squares forming 1 mm^2 were counted (1 square on each quadrant and the central square). If less than 200 SMBs were present per mm^2 (SMB/mm^2), the entire mm^2 was considered. The measurements could not be duplicated because of the limited volume of the samples.

LBC was performed with an automated cell counter Sysmex XT-1800i cell counter (Sysmex Corporation, Kobe, Japan) in the hematology laboratory at Hospital São Lucas. Tracheal samples (25-50 μL) were diluted in a liquefying agent (dithiotreitol) without centrifugation. To perform the LBC, a 10 mg/mL solution of dithiothreitol (DTT; Invitrogen Corporation, Carlsbad, California) in distilled water was prepared in advance and kept frozen at −20°C in Eppendorf tubes until use. Samples were placed in a test tube containing the DTT solution at a ratio of 1 part tracheal aspirate to 6 parts DTT. This sample was vortexed for 10 seconds, and the material was aspirated by the automated Sysmex XT-1800i cell counter (Sysmex Corporation). LBC was performed using the platelet channel. All results obtained were multiplied by 7 to correct for dilution. After specimen collection, patients with acute viral bronchiolitis were followed until PICU discharge or hospital day 28.

Statistical Analyses

The minimum sample size was calculated as 10 patients per group, for an alpha level of 5%, a statistical power of 80%, and expected rates of the factor of interest (surfactant deficiency) of 70% in the exposure group and 10% in the control group.

Asymmetrically distributed data were expressed as medians and IQRs. The Mann–Whitney U and χ^2 tests were used for comparison of continuous and categorical variables respectively. Spearman rank correlation coefficients were used to test for correlation between LBC and SMT results and the ventilator settings and vasoactive drugs duration.

Results

The sample was comprised of 32 patients: 16 in the bronchiolitis group and 16 in the control group (Table). There were no differences between the 2 groups regarding weight, age, or sex. Nine (56.3%) patients in the bronchiolitis group were positive for respiratory syncytial virus, 2 (12.5%) were positive for another virus, and no viruses could be detected in the remaining 5 (31.3%), in whom diagnosis was made on a clinical basis. In the control group, 13 patients (81.3%) were postabdominal surgery status, 1 (6.3%) was postneurosurgery status, 1 (6.3%) was admitted to the PICU for refractory seizures, and 1 (6.3%) had a metabolic disorder. In both groups, most patients (62.5% in the bronchiolitis group and 81.2% in the control group) were born full-term, whereas the remainder were born premature. As expected, peak inspiratory pressure and positive end-expiratory pressure at the time of tracheal aspirate collection were higher in the bronchiolitis group than in the control group (Table).

Median (IQR) LBCs were significantly lower in the bronchiolitis group than in the control group: 130 000 (61 250-362 250) vs 518 000 (180 250-896 000) LBs/μL, $P = .003$ (Figure 1). Likewise, SMB counts were also significantly lower in the bronchiolitis group than in the control group: 10 (2-13) vs 400 (261-615) SMB/mm^2, $P < .001$ (Figure 2). Of the 16 patients with bronchiolitis, 10 (62.5%) had LBCs below the 25th percentile (180 250/μL) observed in the control group. Furthermore, all patients with bronchiolitis had SMB counts below the 25th percentile (261 SMB/mm^2) for the control group.