A Noninvasive Surfactant Adsorption Test Predicting the Need for Surfactant Therapy in Preterm Infants Treated with Continuous Positive Airway Pressure

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Objective To determine the diagnostic accuracy of the surfactant adsorption test (SAT) as a predictor for the need for surfactant replacement therapy in neonates with respiratory distress syndrome (RDS).

Study design Amniotic fluid samples were collected from 41 preterm neonates with RDS treated with continuous positive airway pressure (CPAP) and 15 healthy control term neonates. Purified porcine surfactant served as a further control. Lamellar bodies and lung ultrasound score were also measured in a subset of the neonates treated with CPAP. Surfactant was administered according to the European guidelines, and clinical data were collected prospectively. Surfactant activity was measured as adsorption at the air/liquid interface and given in relative fluorescent units (RFU).

Results Surfactant activity differed among native porcine surfactant (median, 4863 RFU; IQR, 4405-5081 RFU), healthy term neonates (median, 2680 RFU; IQR, 2069-3050 RFU), and preterm neonates with RDS (median, 442 RFU; IQR, 92-920 RFU; $P < .0001$). The neonates who failed CPAP had lower surfactant activity compared with those who did not fail CPAP (median, 92 RFU; IQR, 0-315 RFU vs 749 RFU; IQR, 360-974 RFU; $P = .0002$). Differences between groups were more evident beyond 20-30 minutes of fluorescence; the 30-minute time point showed the highest area under the curve (0.84; $P < .001$) and the best cutoff level (170 RFU; specificity, 72%; sensitivity, 96%) for the prediction of CPAP failure. Surfactant activity at 30 minutes was significantly correlated with lamellar bodies ($r = 0.51$, $P = .006$) and lung ultrasound score ($r = -0.39$, $P = .013$).

Conclusion This technique has the potential to be developed into a fast, simple-to-interpret clinical test. The SAT can reliably identify preterm infants with subsequent CPAP failure and shows promise as a screening test for surfactant replacement in preterm neonates. *(J Pediatr 2017;182:66-73).*

Early continuous positive airway pressure (CPAP) and selective surfactant administration is more effective than surfactant prophylaxis in reducing death and/or bronchopulmonary dysplasia in preterm infants.[1] Based on these data, current American and European guidelines recommend selective surfactant administration after early CPAP.[2,3] Moreover, according to European guidelines, surfactant replacement in preterm neonates treated with CPAP should be given only when oxygen requirements are increasing.[3]

The ideal time for surfactant administration and how to predict which neonates will require surfactant remain unclear. European guidelines recommend surfactant replacement when the fraction of inspired oxygen (FiO_2) rises above 0.3 or 0.4.[3] However, these arbitrary thresholds do not always reflect the true oxygenation status and lung biology during respiratory distress syndrome (RDS).

Some lung maturity tests have been proposed to predict the occurrence of RDS, but these tests measure the amount of surfactant and not its functional activity, and none are widely used in current clinical practice or have been tested to predict CPAP failure in patients with RDS. Maternal lecithin/sphingomyelin ratio or phosphatidylglycerol assays are no longer available, because they are invasive and complex.[4,5] The lamellar body count (LBC) using automated counters is a quick method that appears to be superior to the lecithin/sphingomyelin ratio,[6] but is technically impossible to perform in some cases owing to the thickness of

AF	Amniotic fluid	NICU	Neonatal intensive care unit
AUC	Area under the curve	NS	Natural surfactant
CPAP	Continuous positive airway pressure	OI_{adm}	Oxygenation index
FiO_2	Fraction of inspired oxygen	RDS	Respiratory distress syndrome
GA	Gestational age	RFU	Relative fluorescence units
LBC	Lamellar body count	SAT	Surfactant adsorption test
LUS	Lung ultrasound score		

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tracheal or amniotic fluid. Finally, the stable microbubble test seems to be useful, but is too cumbersome for routine use.[7] A meta-analysis of available studies showed that outcomes are improved following early surfactant replacement, within 2-3 hours after birth.[8] Thus, a reliable tool for predicting the need for surfactant replacement in preterm neonates at an early stage is still needed. Ideally, such a test should be easy to use at the bedside, with quickly available results for rapid and timely decision making.

We recently described a high-throughput method to measure surfactant activity through surface accumulation of fluorescently labeled surfactant.[9] This surfactant adsorption test (SAT) has been successfully used to assay bronchoalveolar lavage fluids from animals and human neonates.[10,11] Because the SAT is a functional test, it may help describe the actual surfactant activity (and lung physiopathology),[9] and thus could be potentially useful for guiding surfactant replacement therapy. The aims of the present study were to evaluate the feasibility of performing the SAT in amniotic fluid (AF) samples and to investigate the usefulness of the SAT as a screening tool for surfactant administration.

Methods

We designed this prospective pilot study to evaluate the diagnostic accuracy of the SAT following Standards for Reporting of Diagnostic Accuracy guidelines.[12] The eligible population consisted of neonates admitted to our neonatal intensive care unit (NICU) between February and June 2015 fulfilling the following criteria: gestational age (GA) <37 weeks and the need for nasal CPAP. Nasal CPAP was started early in the delivery room immediately after stabilization in neonates of ≤32 weeks GA or at NICU admission for RDS in neonates of >32 weeks GA. According to our internal protocol,[13] all infants with signs of respiratory distress received CPAP unless they met 1 or more exclusion criteria. Eligible neonates underwent lung ultrasound in the first 2 hours after NICU admission and before eventual surfactant administration, in accordance with our internal policy. The lung ultrasound score (LUS) was calculated to estimate lung aeration, as described previously.[14] The oxygenation index (OI_{adm}) was calculated at admission as follows: OI_{adm} = CPAP (mm Hg) × FiO_2 × 100/PaO_2. Transcutaneous PO_2 was measured for 15 minutes using a transcutaneous monitor appropriately calibrated at 44°C according to American Association of Respiratory Care guidelines.[15]

Our surfactant administration protocol is based on the 2013 European guidelines.[2] In brief, preterm neonates at ≤32 weeks GA unresponsive to face mask ventilation are intubated at birth[16] and given 200 mg/kg poractant-α (Curosurf; Chiesi Farmaceutici, Parma, Italy) in the delivery room. These neonates were excluded from this study. In all other cases, poractant-α was administered when the FiO_2 requirement reached 0.3 within the first 24 hours of life in infants <28 weeks GA or 0.4 within the first 24 hours of life in infants >28 weeks GA.[2] Late respiratory failures (eg, for sepsis) requiring surfactant were not considered for this study. Protocol compliance was ensured by periodical review and formal retraining; no change in the protocol occurred during the study period. All data were collected prospectively in real time from the NICU electronic database.

A control group of full-term healthy neonates was enrolled with no maternal history of diabetes, cholestasis, or hypothyroidism. These infants had an uncomplicated perinatal transition and early postnatal period and were cared for in the well-baby nursery. Exclusion criteria for both groups were as follows: (1) need for delivery room intubation, according to international guidelines for neonatal resuscitation[16]; (2) meconium aspiration syndrome, defined as the presence of meconium-stained AF and typical chest radiographs or lung ultrasound images[17]; (3) pulmonary hemorrhage, defined as respiratory distress with increasing oxygen requirements, white lung on chest radiographs, and the presence of bloody airway secretions; (4) maternal blood aspiration, defined with the same criteria of pulmonary hemorrhage with the addition of blood-stained AF; (5) antenatal suspicion of any lung malformation; (6) oligohydramnios or anhydramnios; (7) absence of clear AF; and (8) major congenital malformation or known chromosomal abnormalities.

All data were collected prospectively into our NICU electronic database and subsequently analyzed. This study was non-invasive and did not change routine clinical care. The study was approved by the Institutional Review Board, and parental consent was obtained antenatally.

A sample of clear AF (2 mL) was collected at time of vaginal or cesarean delivery and immediately divided into 2 aliquots. One aliquot was used to count lamellar bodies in an automated cell counter, as described previously.[18] The other aliquot was centrifuged at 700 × *g* for 10 minutes, immediately frozen (-35°C), and thawed only once for the analysis. After slow thawing, all samples were briefly vortexed, and phosphatidylcholine concentration was preliminarily assayed using an enzymatic method.[19]

As a second control, we used natural surfactant (NS) obtained by total bronchoalveolar lavage of fresh lungs of slaughtered adult pigs.[20] No pigs were sacrificed for the sole purpose of the study; the use of these animal samples was in accordance with all applicable local regulations. The NS was purified and separated by NaBr density-gradient centrifugation, as described previously.[20] Levels of phospholipids were measured preliminarily through the analysis of lipid phosphorus.[21]

All AF and NS samples were diluted to a concentration of 0.043 μg/μL (corresponding to a total of 3 μg of surfactant in a volume of 70 μL), using 5 mM Tris buffer (pH 7.4, containing 150 mM NaCl). All samples were then stained by incubation with BODIPY-PC (Molecular Probes, Life Technologies, Carlsbad, California) at 37°C for 45 minutes to obtain a final molar ratio of 2% (dye/surfactant). The BODIPY-PC was dissolved into dimethyl sulfoxide to a concentration of 1 μg/μL.

All AF and NS samples were subjected to the SAT, which measures the accumulation of surfactant at the interface, as described by Ravasio et al.[9] This was done in 96-well microtiter plates, with the volume of bulk solution changed from 80 μL to 50 μL in each well. Analysis was performed with a FLUOstar OPTIMA Microplate Reader (BMG Labtech, Offenburg,