Surfactant protein B and A concentrations are increased in neonatal pneumonia

Sara D’Aronco¹, Manuela Simonato¹², Luca Vedovelli², Aldo Baritussio³, Giovanna Verlato¹, Stefano Nobile⁴, Chiara Giorgetti⁴, Matteo Nespeca⁴, Virgilio P. Carnielli⁴ and Paola E. Cogo⁵

**BACKGROUND:** Term newborns with pneumonia show a reduced pulmonary compliance due to multiple and ill-defined factors. Surfactant proteins’ (SPs) changes could have a role in the reduced compliance but the matter is still unsettled. The aim of this study was to clarify the meaning of SPs changes during pneumonia in term newborns.

**METHODS:** In 28 term ventilated newborns, 13 with pneumonia and 15 with no lung disease, we measured SP-B, SP-A, disaturated-phosphatidylcholine (DSPC), and total phospholipids (PL) concentrations in tracheal aspirates at intubation and close to extubation. We also measured DSPC kinetics using (U-13C-PA)dipalmitoyl-phosphatidylcholine.

**RESULTS:** At baseline, SP-B, expressed as % of PL, was significantly different between the groups, being 3.5-fold higher in pneumonia than controls. Conversely, SP-A did not vary between the groups. At extubation, SP-B and SP-A concentrations had decreased significantly in newborns with pneumonia, while there was no significant change in controls. DSPC $t_{1/2}$ was significantly shorter in the pneumonia group (11.8 (5.5–19.8) h vs. 26.6 (19.3–63.6) h, $P = 0.011$).

**CONCLUSION:** In term newborns with pneumonia, SP-B increases with respect to PL, and DSPC is turned over at a faster rate. Disease’s resolution is associated with the restoration of the normal ratio between SP-B and PL.

Alveolar surfactant is essential for lung stability and its composition and functional state are altered during pneumonia and acute respiratory distress syndrome (1,2). Pulmonary surfactant also plays an important role on the innate immune system by enhancing pathogen clearance and by regulating immune-cell functions (3).

Surfactant proteins (SPs) represent less than 10% of total surfactant weight but they play a pivotal role on its function. SP-B is a hydrophobic protein that is considered to be the most important protein for sustaining respiratory physiology (4). SP-B enhances lipid insertion into the monolayer at the air/liquid interface (5), it is involved in the formation of tubular myelin (6), and it may also be part of the host defense mechanisms (7). SP-A, a pulmonary collectin, plays an important role in lung innate immune system (8) but it is also involved in the regulation of surfactant homeostasis and in the formation of tubular myelin (7).

Respiratory failure in newborns with respiratory distress syndrome is due to surfactant deficiency (9), while the reduced pulmonary compliance observed in term newborns at the onset of pneumonia still does not have a clear explanation.

Understanding of SPs changes in children with lung infection is limited and sometimes discordant.

Reduced amounts of SP-A have been found in infants with acute viral bronchiolitis, including forms due to respiratory syncytial virus (10,11) and bacterial and viral pneumonia (12). Kerr et al. (11) found decreased levels of SP-B in the bronchoalveolar lavage fluid (BALF) of children with severe respiratory syncytial virus bronchiolitis, while LeVine et al. (12) found concentrations that did not differ from controls in infants with bacterial and viral pneumonia. Moreover, a recent study in children with acute lung injury, showed no change in either BALF SP-B and SP-A concentrations (13).

In this paper, we aimed to measure SPs changes during pneumonia. We studied term newborns with and without pneumonia who required mechanical ventilation. We compared the concentrations of SP-B and SP-A in tracheal aspirates (TAs) obtained soon after intubation and after clinical improvement just before extubation, in both groups. We also investigated if SP-B and SP-A levels correlate with the degree of respiratory failure and with disaturated-phosphatidylcholine (DSPC) turnover rate, that is known to be increased, with shorter $t_{1/2}$ values, during various acute lung diseases (14,15).

**RESULTS**
We studied 13 newborns with pneumonia and 15 controls with no lung disease. Eight controls were extubated immediately after surgery and therefore, for these patients, we collected only one TA sample.

SP-B, SP-A, DSPC, and total phospholipids (PL) concentrations in epithelial lining fluid (ELF) at the start of the study could be measured in 11 out of 13 newborns with pneumonia and in 14 out of 15 control newborns.
Close-to-extubation, and at least after 6 h from the previous TA, SP-B, SP-A, DSPC, and PL could be measured in 11 out of 13 newborns with pneumonia and in 7 out of 15 controls. At this timepoint, we could not express the amount of SPs as weight/volume of ELF because the arterial line had already been removed.

DSPC kinetic was measured in 19 newborns from whom additional parental consent was obtained, 12 of the pneumonia group and 7 controls. In three newborns (two with pneumonia and one with no lung disease) only DSPC kinetics was measured because the sample was too scarce to perform SPs’ analysis.

Clinical characteristics and respiratory parameters of the study patients, are reported in Table 1. None of the study patients received exogenous surfactant.

The two study groups were comparable for gestational age, birth weight, age, and hours of mechanical ventilation at the study start, while significant differences were observed in C-reactive protein, fraction of inspired oxygen (FiO₂), oxygenation index (OI), alveolar-arterial oxygen gradient (AaDO₂), PaO₂/FiO₂ ratio, peak inspiratory pressure, positive end expiratory pressure, and mean airway pressure (MAP) at the start of the study. A tendency to lower values was observed for PaO₂ in the pneumonia group compared to controls.

By paired analysis, FiO₂, OI, AaDO₂, peak inspiratory pressure, and MAP decreased significantly during the study in the pneumonia group: from 0.50 (0.25–0.60) to 0.25 (0.22–0.29) (P = 0.008); from 5.7 (3.1–7.5) to 2.2 (1.8–2.7) (P = 0.002), from 194.5 (75.6–307.2) to 45.6 (37.9–77.1) (P = 0.006), from 20.8 ± 3.6 to 17.2 ± 2.2 (P = 0.005) cm H₂O, from 8.7 ± 3.1 to 6.1 ± 0.7 (P = 0.003) cm H₂O respectively. PaO₂/FiO₂ increased from 173.9 ± 84.3 to 273.8 ± 89.4 (P = 0.017); no statistical differences were observed in the control group. PaO₂ and positive end expiratory pressure did not change significantly during the study in both groups.

Figure 1 represents changes in OI between the start and the end of the study of all newborns with pneumonia and of those controls who had at least two TA samples.

SP-B, SP-A, DSPC, and PL amounts are reported in Table 2. At the start of the study, SP-B, expressed as percentage of PL (SP-B [%PL]), was significantly higher in pneumonia compared with controls (P = 0.029). We also observed a tendency to a higher ELF SP-B and a lower ELF DSPC and ELF PL.

SPs concentration changes between the first and the last collected TAs, expressed as %PL, are depicted in Figure 2.

In patients with pneumonia SP-B, expressed as % of PL, was significantly higher at the start of the study compared to the percentage found before extubation (Panel a, 0.99 (0.20–1.33)% and 0.05 (0.02–0.10)% respectively, P = 0.003). Similarly, SP-A (%PL) ratio was higher at study start than before extubation (Panel c, 0.59 (0.39–1.23)% and 0.26 (0.13–0.50)% respectively, P = 0.050).

In control newborns, from whom SPs could be measured at least two times (n = 7), SP-B (%PL) did not differ during the study period, (Panel b, 0.23 (0.03–0.31)% and 0.06

---

Table 1. Clinical characteristics and respiratory parameters in pneumonia and control group

|                          | Pneumonia group | Control group | P     |
|--------------------------|-----------------|---------------|-------|
| Gestational agea (weeks) | 38.9 ± 1.4      | 38.6 ± 1.4    | 0.586 |
| Birth weighta (g)        | 3226 ± 605      | 3064 ± 650    | 0.504 |
| Age at study startb (days)| 2.3 (1.0–5.4)   | 2.4 (1.0–8.0) | 0.730 |
| C-reactive protein at study startb (mg/l) | 26.1 (10.4–92.9) | 3.3 (2.9–4.9) | 0.003 |
| Mechanical ventilation at study startb (h) | 44 (12–69)     | 24 (18–52)   | 0.593 |
| FiO₂ at study startb     | 0.50 (0.25–0.60) | 0.21 (0.21–0.23) | 0.001 |
| PaO₂ at study starta     | 67.8 ± 15.4     | 79.7 ± 17.7   | 0.060 |
| OI at study startb       | 5.7 (3.1–7.5)   | 1.4 (1.2–1.7) | <0.001 |
| AaDO₂ at study startb    | 194.5 (75.6–307.2) | 20.1 (16.6–36.6) | <0.001 |
| PaO₂/FiO₂ at study starta| 173.9 ± 84.3    | 361.7 ± 110.6| <0.001 |
| PIP at study startb      | 20.8 ± 3.6      | 15.8 ± 2.4    | 0.001 |
| PEEP at study startb     | 4.0 ± 0.6       | 3.2 ± 0.9     | 0.035 |
| MAP at study startb      | 8.7 ± 3.1       | 5.2 ± 1.2     | <0.001 |

AaDO₂, alveolar-arterial oxygen gradient; FiO₂, fraction of inspired oxygen; MAP, mean airway pressure; OI, oxygenation index; PaO₂, arterial PO₂; PIP, peak inspiratory pressure; PEEP, positive end expiratory pressure.

*Data presented as mean ± SD. †Data presented as median (IQR).
SP-B and SP-A in neonatal pneumonia

DISCUSSION

In this study, SP-B and SP-A amounts in TAs of term newborns with pneumonia were compared with those of newborns with healthy lungs. We also studied the SPs profile during pneumonia clinical course, from the peak of the disease until the clinical improvement and extubation.

Few studies have explored changes in TA SP concentrations during pneumonia in children and no one have presented data corrected for TA dilution (% of PL and ELF) and/or have related the TA SPs amounts to the severity of respiratory failure (12,13).

Table 2. ELF DSPC, PL, SP-B, and SP-A measured at the start of the study; SP-B and SP-A measured at the start of the study and before extubation, expressed as % of PL, in the two study groups

|                      | Pneumonia group | Control group | P |
|----------------------|-----------------|---------------|---|
| ELF DSPC start of the study* (mg/ml) | 1.90 (0.63–4.10) (n = 11) | 4.13 (1.11–5.76) (n = 14) | 0.286 |
| ELF PL start of the study* (mg/ml) | 3.21 (1.41–6.85) (n = 11) | 6.82 (2.25–10.46) (n = 14) | 0.193 |
| ELF SP-B start of the study* (µg/ml) | 22.53 (2.12–56.29) (n = 11) | 7.86 (3.16–23.07) (n = 14) | 0.367 |
| ELF SP-A start of the study* (µg/ml) | 16.99 (11.95–44.19) (n = 11) | 15.99 (11.34–41.48) (n = 14) | 0.815 |
| SP-B (%PL) start of the study* | 0.99 (0.20–1.33) (n = 11) | 0.28 (0.03–0.58) (n = 14) | 0.029 |
| SP-A (%PL) start of the study* | 0.59 (0.39–1.23) (n = 11) | 0.43 (0.23–0.70) (n = 14) | 0.171 |
| SP-B (%PL) pre-extubation* | 0.05 (0.02–0.10) (n = 11) | 0.06 (0.03–0.09) (n = 7) | 0.821 |
| SP-A (%PL) pre-extubation* | 0.26 (0.13–0.50) (n = 11) | 0.40 (0.14–0.79) (n = 7) | 0.497 |

DSPC, disaturated-phosphatidylcholine; ELF, epithelial lining fluid; PL, total phospholipids.
*Data presented as median (IQR).

Figure 2. Changes in surfactant protein (SP) (%PL) ratio in tracheal aspirates collected at the start of the study and close to the extubation in the two study groups. Bold lines represented the intersection of the two median SP (%PL) ratio values of each group. Panels a and c refer to pneumonia group, b and d to control group. *P ≤ 0.05.
Expressing SPs levels as % of PL, we found that in patients with pneumonia, SP-B was significantly higher than in controls at study start and that both SP-B and SP-A decreased, as pulmonary function improved. Expressing data as µg/ml ELF, we found no significant differences between pneumonia and control patients, although the levels of SP-B tended to be higher and those of DSPC and PL tended to be lower at the start of the study in the pneumonia group. We also found that DSPC was turned over at a faster rate in pneumonia and that DSPC PS was significantly decreased in the pneumonia group.

Based on literature data (9,16,17), we expected to find lower levels of SP-B at the onset of pneumonia and to observe increasing SP-B levels during recovery. Despite the heterogeneity of data, we were instead struck by finding at the onset of pneumonia an excess of SP-B, with respect to PL. It is possible that SP-B excess represents "spent surfactant", that could contribute with factors, like a leak of plasma proteins in the alveolar space (2), or SP-C deficiency (18), to the decreased lung compliance observed at the onset of the pneumonia. The observed SP-B excess could, however, be explained by other mechanisms like increased secretion of SP-B, a slower clearance or a combination of these mechanisms. Finally, it is possible that the excess of SP-B may reflect an accumulation of surfactant subfractions particularly enriched in SP-B (19). The decrease of the SP-B (%PL) ratio along with clinical and respiratory improvements, were reasonably related with a better oxygenation status, which may represent the result of an improved clearance or a decreased secretion of SP-B. Our data are in line with previous results obtained both in animal studies (20,21) and in newborns after lung injury (22,23).

Ikegami et al. (20) found that in healthy mice (with normal lungs) the induction of lung injury by the intratracheal injection of LPS resulted in an increase of DSPC and SP-B, mediated by STAT-3, which is consistent with an acute response to the lung injury. Moreover, our research group observed how, in a murine model of unilateral lung injury, DSPC-palmitate synthesis was faster both in the injured and in the noninjured lungs, compared to the naïve control lungs. In this way we proved that, after a local instillation of acid in one bronchus, it is the entire lung system that is involved in responding to the damage and not only a local area of the lung (21). Other mechanisms may have contributed to the elevation of SP-B levels. Increased expression of SPs has been observed both in animals exposed to high levels of inspired oxygen (24) and in mechanically ventilated ones (25), however we hypothesised that a main role could have been played by the infection and be mediated by STAT-3 pathway.

A study performed by Epaud et al. (26) found that elevated levels of mature SP-B peptide in the airspaces of transgenic mice were associated with decreased inflammation following exposure to endotoxins. Based on these findings, a larger study could help us to understand if, in term newborns with pneumonia, higher levels of SP-B are associated with a better clinical outcome and a faster recovery.

The decreased SP-A (%PL) ratio during resolution of pneumonia could be related either to differences in the turnover of surfactant or to other phenomena related to the nonrespiratory properties of SP-A.

Only two reports have previously described surfactant composition changes during bacterial pneumonia in infants (12,13). Both studies corrected the TA dilution by TA total protein amount; this method is less reliable than the ELF method because of the risk to underestimate the SPs amounts due to the increased alveolar capillary leak during the disease's course (27). Kerr et al. (11) measured SP levels in infants ventilated for respiratory failure resulting from severe respiratory syncytial virus bronchiolitis but TA dilution was, also in this case, corrected by TA total protein amount.

In our study, we used urea to correct for surfactant dilution (28) in the first TA sample, and we expressed SPs concentrations as percentage by weight of total PL in absence of available plasmatic urea level in the second TA. At the end of the study, after the removal of the arterial line, we could not express SPs as ELF, because ethical concerns precluded invasive and unnecessary procedures, such as the collection of plasma to correct for surfactant dilution. We therefore chose to express SPs values as % of PL because the amount of total PL recovered from TAs did not significantly differ from the start to the end of the study both in the pneumonia and in the control groups (pneumonia: 0.14 (0.07–0.25) and 0.17 (0.09–0.97) mg/ml, P = 0.110; controls: 0.50 (0.20–0.75) and 0.39 (0.06–0.56) mg/ml, P = 0.237). This implied that they did not affect the SPs (%PL) ratio and they did not introduce a bias in the comparison within the study groups. A previous study performed in adults with pneumonia, ARDS, or cardiogenic edema showed that in adults with pneumonia the BALF PL and phosphatidylcholine (PC) content were not significantly different compared with controls (2). It is conceivable that the TAs unsaturated-PC derived from inflammatory cells may have contributed to maintain the same amount of TAs PL content in our newborns with pneumonia together with the increase of other surfactant PL classes, either than the sole DSPC (13). Regarding SP-A (%PL) ratio, Dargentville et al. (10) expressed SP-A content as weight/volume of ELF, and as opposed to our study, they found that ELF SP-A in infants with respiratory syncytial virus bronchiolitis was, at the peak of the disease, significantly lower compared to controls. This conflicting finding can be explained by the different etiology of the disease or by the fact that they measured ELF SP-A in infants with gestational age ranging between 24–41 wk.

The administration of a tracer dose of (U-13C-PA)dipalmitoyl-phosphatidylcholine allowed us to measure the DSPC kinetics, confirming previous findings of our research group (15). We found that in newborns with pneumonia DSPC PS and t½ are, respectively, lower and shorter compared to controls. The faster turnover of TA DSPC in these patients likely reflects the hyperventilation associated with pneumonia and/or the increased DSPC synthesis. The increased catabolism of DSPC observed in children with pneumonia supports the hypothesis that increasing SP-B levels could represent a compensatory mechanisms of the lung to a damage, as we earlier described in a murine model of unilateral lung injury (21).
Our study has four major limitations: first, subject number; second, term newborns who served as controls were on mechanical ventilation for a median value of 24 h, which may have altered the surfactant homeostasis, hiding possible significant differences between healthy and ill newborns; third, ethical limitations prevented the plasma collection close to the extubation and the calculation of SPs in ELF. Finally, to measure DSPC kinetics, we used (U-13C-PA)dipalmitoyl-phosphatidylcholine as tracer, in agreement with previous animal studies (29) but without the possibility to prove the actual mixing with the endogenous surfactant pool.

In conclusion, in term newborns with pneumonia SP-B increased with respect to PL and DSPC turned over at a faster rate. Resolution of the disease was associated with restoration of the normal ratio between SP-B and PL.

METHODS

Study Population
From January 2011 to December 2013, we prospectively recruited 13 term newborns (37–41 wk GA), up to 10 d of life with neonatal pneumonia. We also recruited 15 newborns with no lung disease who required mechanical ventilation for elective surgery or neurological impairment. All newborns were admitted to the Neonatal Intensive Care Units of the University of Padua or of the Polytechnic University of Marche, Ancona, Italy.

The diagnosis of pneumonia was based on the 2008 CDC/NHSN (Centers for Disease Control and Prevention/National Healthcare Safety Network) criteria for pneumonia in infants ≤1 y of age (30). These criteria are basically limited to clinically defined pneumonia and are: (i) Chest x-ray showing new or progressive or persistent infiltrate, consolidation or cavitation or pneumatoceles; (ii) Worsening gas exchange (desaturation or rise in oxygen requirement or rise in ventilation demand); (iii) At least three of the following: temperature instability with no other recognized cause; leucopenia (<4,000 WBC/mm³), or leucocytosis (>15,000 WBC/mm³) with left shift (>10% band cells); new onset of purulent sputum, or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements; apnea, tachypnea, nasal flaring with retraction of chest wall or grunting; wheezing, rales or rhonchi; cough; bradycardia (<100 beats/min) or tachycardia (>170 beats/min). Diagnosis of bacterial pneumonia was based on these indirect clinical criteria and on the response given by every infant to the antibiotic therapy.

Inclusion criteria were: respiratory failure (newborns with pneumonia) requiring mechanical ventilation with a FiO₂ > 0.35, a mean airway pressure > 7 cm H₂O, and a prediction to be mechanically ventilated for at least 48 h. Control newborns had no lung disease (no clinical and laboratory signs of infection, normal chest x-ray, FiO₂ < 0.30) but required mechanical ventilation as a result of major surgery or neurological impairment leading to poor airway control.

Another inclusion criterion for both groups was the presence, at the start of the study, of an arterial line, placed for invasive arterial blood gas analysis.

Statistical analysis was performed using PASW Statistics 18.0 for Windows (SPSS, Chicago, IL.).

STATEMENT OF FINANCIAL SUPPORT
No financial support was receveid for this study.

Disclosure: The authors confirm that there is no potential, perceived, or real conflict of interest.

REFERENCES
1. Gregory TJ, Longmore WJ, Mosley MA, et al. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. J Clin Invest 1991;88:1976–81.
2. Günther A, Siebert C, Schmidt R, et al. Surfactant alterations in severe pneumonia, acute respiratory distress syndrome, and cardiogenic lung edema. Am J Respir Crit Care Med 1996;153:176–84.

Copyright © 2015 International Pediatric Research Foundation, Inc.

Volume 78  |  Number 4  |  October 2015  Pediatric RESEARCH 405
3. Kuroki Y, Takahashi M, Nishitani C. Pulmonary collectins in innate immunity of the lung. Cell Microbiol 2007;9:1871–9.
4. Pérez-Gil J. Structure of pulmonary surfactant membranes and films: the role of proteins and lipid-protein interactions. Biochim Biophys Acta 2008;1778:1676–95.
5. Hawgood S, Derrick M, Poulain F. Structure and properties of surfactant protein B. Biochim Biophys Acta 1998;1408:150–60.
6. Clark JC, Muster SE, Bachurski CJ, et al. Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newborn mice. Proc Natl Acad Sci USA 1995;92:7794–8.
7. Haagsman HP, Diemel RV. Surfactant-associated proteins: functions and structural variation. Comp Biochem Physiol A Mol Integr Physiol 2001;129:911–108.
8. LeVine AM, Whitsett JA. Pulmonary collectins and innate host defense of the lung. Microbes Infect 2001;3:161–6.
9. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. AMA J Dis Child 1959;97(5, Part 1):517–23.
10. Dargaville PA, South M, McDougall PN. Surfactant abnormalities in infants with severe viral bronchiolitis. Arch Dis Child 1996;75:133–6.
11. Kerr MH, Paton JY. Surfactant protein levels in severe respiratory syncytial virus infection. Am J Respir Crit Care Med 1999;159(4 Pt 1):1115–8.
12. LeVine AM, Lotze A, Stanley S, et al. Surfactant content in children with inflammatory lung disease. Crit Care Med 1996;24:1062–7.
13. Todd DA, Marsh MJ, George A, et al. Surfactant phospholipids, surfactant proteins, and inflammatory markers during acute lung injury in children. Pediatr Crit Care Med 2010;11:82–91.
14. Cogo PE, Toffolo GM, Ori C, et al. Surfactant disaturated-phosphatidylcholine kinetics in acute respiratory distress syndrome by stable isotopes and a two compartment model. Respir Res 2007;8:13.
15. Facco M, Nespeca M, Simonato M, et al. In vivo effect of pneumonia on surfactant disaturated-phosphatidylcholine kinetics in newborn infants. PLoS One 2014;9:e93612.
16. Nogee LM. Genetics of the hydrophobic surfactant proteins. Biochim Biophys Acta 1998;1408:323–33.
17. Nogee LM, de Mello DE, Dehner LP, Colten HR. Brief report: deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. N Engl J Med 1993;328:406–10.
18. Mulugueta S, Beers MF. Surfactant protein C: its unique properties and emerging immunomodulatory role in the lung. Microbes Infect 2006;8:2317–23.
19. Baritussio A, Alberti A, Quaglino D, et al. SP-A, SP-B, and SP-C in surfactant subtypes around birth: reexamination of alveolar life cycle of surfactant. Am J Physiol 1994;266(4 Pt 1):L436–47.
20. Iekami M, Falcione A, Whitsett JA. STAT-3 regulates surfactant phospholipid homeostasis in normal lung and during endotoxin-mediated lung injury. J Appl Physiol (1985) 2008;104:1753–60.
21. Lamonica G, Amigoni M, Vedovelli L, et al. Pulmonary surfactant synthesis after unilateral lung injury in mice. J Appl Physiol (1985) 2014;116:210–5.