# Is there a necessity for multiple doses of surfactant for respiratory distress syndrome of premature infants?

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**SUMMARY: Tsakalidis C, Giougki E, Karagianni P, Dokos C, Rallis D, Nikolaidis N. Is there a necessity for multiple doses of surfactant for respiratory distress syndrome of premature infants? Turk J Pediatr 2012; 54: 368-375.**

**Both prophylactic and early surfactant (SF) replacement therapy reduce pulmonary complications and mortality in ventilated infants with respiratory distress syndrome (RDS). The effectiveness of one or more doses and the impact on morbidity and mortality of premature neonates with RDS need to be further clarified. The objective of this study was to investigate the necessity of repeated surfactant replacement therapy in premature infants ≤32 weeks of gestational age and the possibility of an underlying pathology. This study included 126 premature neonates of 24–32 weeks of gestation. We used 200 mg/kg per dose of porcine surfactant (Curosurf®) as primary treatment and 100 mg/kg in cases that required retreatment. The subjects were classified into two groups: the first group (Group 1) received a single dose of surfactant (n=98) and the second group (Group 2) included infants who required more than one dose (n=28). The 1st dose was administered in the first 20 minutes after birth while the second was given six hours later. In four cases, a 3rd dose was required, that was provided 12 hours after birth. Recorded data included: clinical and radiological classification of RDS, extubation time, oxygenation estimation indexes (OI: oxygenation index, A-a$DO_2$: alveolar-arterial oxygen difference, a/$APO_2$: arterial-alveolar ratio of partial oxygen pressure), requirement and duration of oxygen administration, total duration of mechanical ventilation, and survival rate. Patient Group 1 did not present any radiological findings of RDS of grade 3 or 4 six hours after SF administration, whereas such findings were recorded in three neonates of Group 2. Therefore, we assumed that failure of a single-dosing treatment indicates a more severe RDS and might reflect an underlying pathology. The impact of maternal chorioamnionitis in the neonates that necessitated further replacement therapy was statistically significant (p=0.045); moreover, infection markers were positive in the majority of the patient population of the second group. Twenty-two neonates (22%) of the first group needed intubation in the delivery room compared to 16 (57%) of the second group (p=0.0001). In conclusion, premature infants treated with a single dose of surfactant can usually be successfully extubated. Requirement of retreatment could be attributed to other pathogenetic mechanisms. A positive history of maternal chorioamnionitis was the commonest reason.**

***Key words: pulmonary surfactant, respiratory distress syndrome, newborn, prematurity.***

Respiratory distress syndrome (RDS) occurs primarily in premature infants, with an incidence that is inversely proportional to gestational age (GA) and birth weight (BW); 60–80% of infants with GA of <28 weeks and 30% of those with GA of 32–36 weeks develop RDS[1,2]. RDS is associated with a 30% mortality rate in the neonatal population[1].

Nearly 50 years after the discovery that infants with RDS are deficient in pulmonary surfactant (SF) and more than 10 years after the introduction of exogenous SF for routine clinical use in newborns, questions remain concerning the optimal treatment strategy. Multiple randomized controlled trials have demonstrated that both prophylactic and

rescue treatment are safe and effective, as demonstrated by a reduction in oxygen and ventilatory requirements[2-5]. However, SF therapy at less than six hours (6 h) of age is effective in acutely reducing oxygen and ventilatory requirements in premature infants. In cases of established RDS, SF treatment results in reduced ventilatory requirement[6] and lower incidence of pulmonary interstitial emphysema[7-12], pneumothorax[7-9,11-15] and chronic lung disease[8-12]. Consequently, SF therapy can reduce RDS mortality by approximately 40%[1-3,7,8,10,12].

Although the effect of one dose was frequently sustained, some neonates exhibited deterioration in lung function after the initial positive response. In the human SF trials in which additional doses were allowed, 22 of 31 infants in the study of Merritt et al.[8], 9 of 22 in that of Hallman et al.[9] and 8 of 10 infants from the study of Lang and coworkers[5] met the criteria for retreatment and were given one or more extra doses. Many of them responded to additional doses. Recent data on the effects of bovine SF in neonates of >30 weeks of gestation with moderately severe RDS (arterial-alveolar ratio of partial oxygen pressure [a/$APO_2$] ~ 0.30) suggest that a single dose of 100 mg/kg is insufficient in many cases and that babies who relapse may benefit from multiple doses[15]. There is also strong evidence that the incidence of air leak complications and neonatal death in babies with severe RDS can be further reduced by using multiple doses of porcine SF (Curosurf®). The multiple-dose regimen may also increase the pool size of alveolar SF lipids available for recycling during the recovery phase of the disease[6].

Kendig et al.[16] observed that many neonates treated with calf lung SF extract at birth show only transient response. The study of Shapiro et al.[17] documented that multiple doses of SF may help sustain the primary effect. Konishi et al.[18] in their study examining SF dosage also allowed extra doses to be supplied. The European Multicenter Trial presented a reduction in mortality and bronchopulmonary dysplasia (BPD) from 45% in the single-dose group to 25% in the multiple-dose group[11].

The present study focuses on determining the specific characteristics of infants aged ≤32 weeks of GA for whom a single dose of SF was insufficient. The possible beneficial effect of additional doses on oxygenation was also evaluated. A suboptimal response to the 1st dose is examined as an indicator of a possible underlying pathology.

## Material and Methods

### *Characterization of Surfactant*

Curosurf® is a preparation of polar lipids isolated from minced pig lungs by a combination of washing, centrifugation and extraction with chloroform-methanol and liquid-gel chromatography. It contains approximately 99% lipids, mainly phospholipids, and 1% hydrophilic proteins of low molecular weight (SP-B and SP-C). In a pulsating bubble system at 37°C, Curosurf® (diluted to 10 mg/ml) has a minimum surface tension of 0 mN/m within 10 minutes (min) of area oscillation[19]. Tracheal instillation of Curosurf® (160 mg/kg) led to a striking improvement in lung function both in immature newborn rabbits and in SF-depleted adult animals[20-22].

### *Study Design and Setting*

We conducted a retrospective analysis of preterm neonates that received a single or repeated SF treatment, according to their requirements. All infant subjects were born in our hospital between May 2004 and January 2007. Maternal written consent was obligatory in order for the infants to enter the study, according to the requirements of the Ethics Committee.

Our study included 126 premature neonates of 24–32 weeks of GA. GA was based on the last menstrual period, on ultrasonographic determinations between the 8th and 14th weeks of gestation and on the Ballard test in cases of unknown GA. Mechanical equipment included Babylog 8000 plus ventilators (Drager) and nasal continuous positive airway pressure (NCPAP) devices (Medin). The subjects were classified into two groups: Group 1was treated with a single dose of 200 mg/kg of SF (n=98) and Group 2 with more than one dose of 100 mg/kg (n=28).

All infants were electively intubated for administration of 200 mg/kg porcine isolated SF (Curosurf®, Chiesi Farmaceutici SPA,

Parma, Italy) as soon as practicably possible (within 20 min after birth) and NCPAP was then initiated. Liveborn infants ≤27 weeks GA received prophylactic SF within 15 min after birth. In infants at risk for RDS with GA of 28-32 weeks, SF was administered if a fraction of inspired oxygen ($FiO_2$) ≥40 was needed to reach oxygen saturation ($SpO_2$) between 85-93% or the infant exhibited signs of moderate to severe respiratory distress at age 20 min (early treatment). SF was instilled as bolus into the main bronchus, via the endotracheal tube through a no. 5 feeding catheter with the neonate lying supine. Following each SF instillation, the infants were ventilated manually for 1 min using the same $FiO_2$ as before the replacement maneuver. Infants who received SF either prophylactically or as early rescue treatment were then switched to NCPAP usually immediately or as soon as possible depending on their respiratory condition. The initial pressure of CPAP was 6-8 cm $H_2O$ or more, aiming to recruit the maximum number of alveoli. A chest X-ray was also performed.

Criteria for administration of additional doses of SF were: 1) clinical and radiological findings typical of RDS, 2) a positive response to the 1st dose of SF with a decrease in $FiO_2$ by at least 0.1 and/or down to 0.2, and 3) a respiratory deterioration signaled by a continuing requirement of mechanical ventilation with a $FiO_2$ >0.4 that was not caused by pneumothorax. These infants were mechanically ventilated with a $FiO_2$ >0.4, a frequency of 30-50 breaths per min, a peak inspiratory pressure (PIP) of <25 cm $H_20$, a positive end-expiratory pressure (PEEP) of 5-7 cm $H_20$, and an inspiration to expiration ratio of 1:2 to 1:3. Infants who achieved retreatment criteria as outlined above received 100 mg/kg of SF in the same way. Then, the infants were reconnected to the ventilator, and the $FiO_2$ and ventilator settings were immediately adjusted to the infants' clinical response, in order to maintain adequate blood gas values (partial pressure of oxygen in arterial blood $(Pa)O_2$: 50-70 mmHg, $PaCO_2$: 40-45 mmHg, pH >7.3) with the lowest possible levels of $FiO_2$ and PIP. The 2nd dose of SF was administered 6 h after the first. In four cases, a 3rd dose was required, which was provided 12 h after birth.

Classification of pulmonary X-ray findings for RDS includes: Grade 1: slight reticular (slight granular) decrease in transparency of the lung with no certain difference from normal findings; Grade 2: soft decrease in transparency with an air-bronchogram, which overlaps the heart; Grade 3: gradual stronger decrease in transparency, as well as a blurry diaphragm and heart; and Grade 4: practically homogenic lung opacity[23].

Preconditions for SF instillation were absence of acidosis, hypotension and hypothermia. No suctioning of the airways was performed during the first 6 h after each SF instillation. Infants were not enrolled if there was evidence of prolonged rupture of membranes (≥3 weeks), intraventricular hemorrhage of grade III or IV, birth asphyxia (defined as Apgar score ≤3 at 5 min, umbilical arterial cord pH <7.1, early onset of seizures), or major congenital anomalies (e.g. severe cardiovascular malformations, myelomeningocele, chromosomal aberrations, Potter's syndrome).

Arterial blood gas values were determined at regular intervals: 0, 3, 6, 12, 24, 48, 72, and 96 h following the 1st dose of SF in both groups. Recorded data included: clinical and radiological classification of RDS, extubation time, oxygenation estimation indexes (OI: oxygenation index, A-$aDO_2$: alveolar-arterial oxygen difference, $a/APO_2$), requirement and duration of oxygen administration, total duration of mechanical ventilation, and survival rate. Chest roentgenograms were obtained at 6 h after each dose of SF.

## Statistics

All data recorded were summarized using descriptive statistical methods. Summary statistics were presented as mean ± standard deviation. Comparison of mean values of the parameters was performed using unpaired Student's t-test. The level of statistical significance was set as $p \leq 0.05$. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) program (11.0 for Windows software, LEAD Technology, Inc).

## Results

A total of 126 premature neonates of 24-32 weeks GA were judged eligible to enter the

**Table I.** Demographic Characteristics and Data on Perinatal History of the Two Groups

| | Neonates that received 1 dose of SF | Neonates that received >1 dose of SF | p |
|---|---|---|---|
| Total (n) | 98 | 28 | |
| BW (g, x ± SD) | 1323 ± 349 | 1128 ± 375 | 0.018 |
| GA (w, x ± SD) | 30 ± 2 | 28 ± 2 | 0.001 |
| Male gender [n (%)] | 53 (54) | 12 (43) | 0.42 |
| In vitro fertilization [n (%)] | 49 (50) | 9 (32) | 0.18 |
| Steroids prenatally [n (%)] | 85 (87) | 23 (82) | 0.79 |
| RoM >24 h [n (%)] | 17 (17) | 5 (18) | 0.95 |
| Chorioamnionitis [n (%)] | 14 (14) | 8 (29) | 0.045 |
| Cesarian section [n (%)] | 96 (98) | 28 (100) | 0.91 |
| Apgar score <5 [n (%)] in 1 | 5 (5) | 2 (7) | 0.63 |
| Intubation at labor | 22 (22) | 16 (57) | 0.0001 |

study; the single-dose group included 98 and the multiple-dose group 28 neonates. Demographic characteristics of the subjects are outlined in Table I. The infants of Group 2 presented a statistically significant decrease in BW (p=0.018) and GA (p=0.001), and an increase in requirement for intubation at labor (p=0.0001), as well as maternal history of chorioamnionitis (p=0.045). Moreover, the infection markers were positive in the majority of the patient population of Group 2. Twenty-two neonates (22%) of Group 1 needed intubation in the delivery room compared to 16 (57%) of Group 2 ($p$=0.0001).

The outcome of management of both groups is presented in Table II. Only 2 (2%) infants of Group 1 had radiological findings of 1st and 2nd grade RDS at 6 h following birth in contrast to 12 (43%) of Group 2. There were no radiological findings of RDS of grade 3 or 4, 6 h after SF administration in Group 1, whereas such findings were recorded in 3 neonates of Group 2. $FiO_2$ requirements presented a descending course at 6, 12 and

**Table II.** Outcome of the Studied Infants

| | Neonates that received 1 dose of SF | Neonates that received >1 dose of SF | p | Total |
|---|---|---|---|---|
| N | 98 | 28 | | 126 |
| RDS 1st-2nd degree radiologically at 6 h [n (%)] | 2 (2) | 12 (43) | <.0.0001 | 14 (11) |
| RDS 3rd-4th degree radiologically at 6 h [n (%)] | 0 (0) | 3 (11) | 0.001 | 3 (2) |
| $FiO_2$ at 6 h (x±SD, range)* | 0.24 ± 0.05 (0.21-0.4) | 0.46 ± 0.28 (0.21–1) | 0.001 | 0.29 ± 0.17 (0.21– 1) |
| $FiO_2$ at 12 h (x±SD, range)* | 0.23 ± 0.04 (0.21-0.4) | 0.42 ± 0.24 (0.21-1) | 0.0005 | 0.27 ± 0.14 (0.21–1) |
| $FiO_2$ at 24 h (x±SD, range)* | 0.23 ± 0.05 (0.21-0.5) | 0.36 ± 0.19 (0.21-0.95) | 0.003 | 0.26 ± 0.11 (0.21–0.95) |
| Total duration of $O_2$ administration (d, x±SD, range)* | 13.7 ± 22.4 (0-109) | 31.46 ± 37.9 (0-139) | 0.02 | 16.31 ± 26.5 (0-139) |
| Duration of MV after the prophylactic administration (h, x±SD, range)* | 9.47 ± 22.6 (0-120) | 92.88 ± 82.1 (0–336) | <0.0001 | 26.22 ± 53.2 (0–336) |
| Total duration of MV (h, x±SD, range)* | 33.97± 62.7 (0-426) | 315.07 ± 559.8 (0-2016) | 0.02 | 91.55 ± 282.1 (0–2016) |
| Deaths [n (%)] | 1 (1) | 3 (11) | <0.0001 | 4 (3) |
| Causes of death | | | | |
| Sepsis [n (%)] † | 1 (100) | 2 (66) | | 3 (75) |
| Intrauterine stress [n (%)] † | 0 (0) | 1 (33) | | 1 (25) |

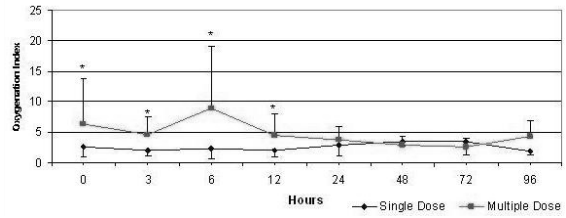


**Fig. 1.** Oxygenation Index of the two groups at 0 (p=0.019), 3 (p=0.004), 6 (p=0.02), 12 (p=0.02), 24 (p=0.4), 48 (p=0.3), 72 (p=0.5), and 96 hours (p=0.07) after SF instillation.

24 h following SF instillation in both groups, although in significantly higher levels in the retreated infants (p=0.001, 0.0005 and 0.003, respectively). Duration of mechanical ventilation after prophylactic SF administration as well as the total time on mechanical ventilation were significantly reduced in the single-dose group (p<0.0001 and p=0.02, respectively). Four deaths occurred, 1 in Group 1 due to septicemia and 3 in Group 2; 2 of them were attributed to septicemia in the field of maternal chorioamnionitis and 1 in intrauterine stress.

Alterations in OI are shown in Figure 1; at time point 0, there was a statistically significant difference between the two groups ($p$=0.019) that remained at 3, 6 and 12 h (p=0.04, p=0.02 and p=0.02, respectively) after SF installation. Changes in oxygenation as judged by A-a$DO_2$ and a/$APO_2$ are presented in Figures 2 and 3, respectively; both A-a$DO_2$ and a/$APO_2$ differed significantly, showing a better oxygenation during the first 48 h following SF treatment in the single-dose group. Oxygenation was improved in both groups at 48 h after treatment. Figure 4 shows the percentage of subjects of each group that needed mechanical ventilation after treatment at each time point following treatment. Ventilatory requirements were significantly lower in the infants that were successfully weaned after receiving the initial dose of SF. All results are clearly in favor of the single-dose SF group.

## Discussion

Respiratory distress caused by SF deficiency remains the most common cause of death and handicap in premature infants[24]. Improved survival occurred with the introduction of mechanical ventilation, although it may lead to significant complications such as pulmonary injury and BPD[6]. The introduction of natural SFs for the treatment of established RDS was a major breakthrough in neonatal medicine, as SF replacement therapy has shown great promise in alleviating the lung immaturity of very low birth weight neonates. The goal of therapy is to maintain minute volume by maintaining functional residual capacity and open alveoli for gas exchange.

Administration of SF is beneficial both in prophylactic and therapeutic management of RDS[3-5]; however, there is a strong rationale to support the preference for prophylactic or early treatment over rescue SF treatment, with improved outcomes in high-risk preterm infants[24,25]. Preventive treatment offers the theoretical advantage of replacing SF before the onset of respiratory disease, decreasing the need for ventilator support and therefore avoiding secondary barotraumas that may result from even short periods of assisted ventilation[26,27]. In animal models, SF is distributed more homogeneously when given immediately after birth into lungs that are still fluid-filled[28]. Besides the starting time of therapy, the efficacy of SF administration has been associated directly with GA, BW[29-33], single pregnancy, a history of maternal steroid therapy prior to delivery, and an Apgar score >7 at 1 and 5 minutes[33-37].

Our data indicate that neonates with lower BW and GA are candidates for repeated SF treatment, a conclusion that is explained by their increased respiratory immaturity. At 6 h after SF instillation, there were more cases of established RDS of 1st -2nd degree as well as of 3rd-4th degree in the retreated infants (documented by roentgenogram). Obviously, the higher incidence and severity of RDS in

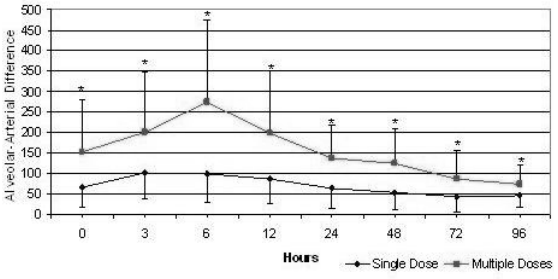


**Fig. 2.** Alveolar-arterial oxygen difference of the two groups at 0 (p=0.001), 3 (p=0.004), 6 (p=0.001), 12 (p=0.001), 24 (p<0.0001), 48 (p=0.0002), 72 (p=0.005), and 96 hours (p=0.004) after SF instillation.

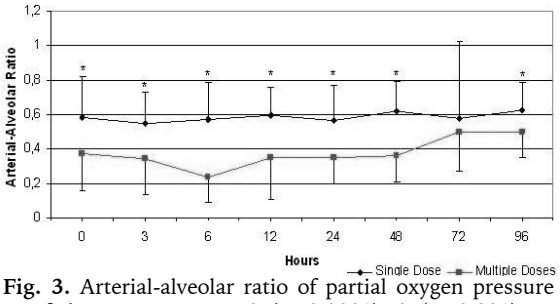


**Fig. 3.** Arterial-alveolar ratio of partial oxygen pressure of the two groups at 0 ($p<0.0001$), 3 ($p=0.001$), 6 ($p<0.0001$), 12 ($p<0.0001$), 24 ($p<0.0001$), 48 ($p<0.0001$), 72 ($p=0.23$), and 96 hours ($p<0.0009$) after SF instillation.

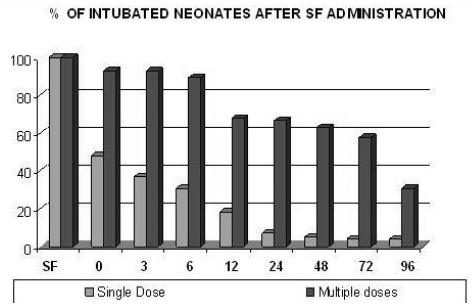


**Fig. 4.** Percentage of infants of the two groups that required mechanical ventilation at 0, 3, 6, 12, 24, 48, 72, and 96 hours after SF installation.

the first hours of life predispose to repeated SF treatment.

Requirement for intubation during resuscitation of premature neonates seems to be a statistically important risk factor for the need of additional SF doses, since perinatal stress inactivates SF. Irrespective of whether the infants received single- or multiple-dose treatment, administration of the initial dose of SF resulted in rapid improvement of gas exchange, as reflected by a significant improvement in A-a$DO_2$ and a/$APO_2$ ratios, and $FiO_2$ could be rapidly reduced in both groups. Therefore, it is obvious that failure of a single SF dose might indicate more severe RDS. The duration of mechanical ventilation was significantly increased in the infants that required more than one dose, reflecting the severity of RDS. However, this observation is not in agreement with the meta-analysis of Soll and Özek[38], possibly because of the differences in the retreatment criteria between their study and ours.

Oxygenation indexes presented significant differences between the groups: values of OI were higher at 3, 6 and 12 h following SF instillation in the infants that were retreated. As expected, levels of A-a$DO_2$ and a/$APO_2$ reflected the same pattern. Moreover, the single-dosing group required lower levels and less time of supplemental $O_2$. Improvement in oxygenation requirements was seen in both groups 48 h following treatment, presumably reflecting endogenous SF production.

Statistical analysis revealed an important increase in the incidence of maternal chorioamnionitis in the subjects of Group 2; this finding is in accordance with literature data that indicate a higher incidence of RDS in premature infants whose mother had established chorioamnionitis[39]. Such infants often necessitate SF retreatment. Moreover, infants with pulmonary infection due to group B streptococcus, a very common vaginal pathogen, require increased doses of SF compared to infants with RDS[40].

In conclusion, comparative evaluation of the two groups demonstrated that despite the difference in GA and therefore in the severity of RDS, the infants who required retreatment presented a higher incidence of maternal chorioamnionitis. Literature data indicate that a newborn with severe RDS may necessitate multiple SF dosing. However, failure of successful extubation after the first administration of SF and requirement of more doses could be a strong indication of a possible underlying pathology, such as intrauterine infection.

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