Surfactant Protein D in Newborn Infants: Factors Influencing Surfactant Protein D Levels in Umbilical Cord Blood and Capillary Blood

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ABSTRACT

Surfactant protein D (SP-D) is a collectin that plays an important role in the innate immune system. The role of SP-D in the metabolism of surfactant is as yet quite unclear. The aims of this study were to establish normal values of SP-D in the umbilical cord blood and capillary blood of mature newborn infants and to assess the influence of perinatal conditions on these levels. A total of 458 infants were enrolled in the present study. Umbilical cord blood was drawn at the time of birth and capillary blood at age 4 to 10 d. The concentration of SP-D in umbilical cord blood and capillary blood was measured by enzyme-linked immunosorbent assay. The median concentration of SP-D in umbilical cord blood was 392.1 ng/mL and was found to be influenced by maternal smoking and labor. The median

concentration of SP-D in capillary blood was 777.5 ng/mL and was found to be influenced by the mode of delivery, the highest levels being observed in infants born by cesarean section. It was concluded that SP-D concentrations in umbilical cord blood and capillary blood are highly variable and depend on several perinatal conditions. Further studies are needed to elucidate the effect of respiratory distress and infection on SP-D concentrations. (*Pediatr Res* 58: 908–912, 2005)

Abbreviations

MBL, mannan-binding lectin
SP, surfactant protein
SP-D, surfactant protein D

Pulmonary surfactant is synthesized and secreted by alveolar type II epithelial cells and consists of approximately 90% lipid and 5–10% proteins (1). Four proteins, called SP-A to -D, have been identified. SP-B and SP-C have been characterized as hydrophobic polypeptides that enhance the adsorption of lipid to the surface of the alveoli, whereas SP-A and SP-D are hydrophilic and participate in the innate host defense immune system (2–4). SP-A and SP-D belong to the group of collectins, which are structurally very similar to complement protein C1q (5).

Collectins are oligomeric molecules consisting of carbohydrate recognition domains attached to collagen-like regions (5). In humans, three well-studied collectins are known at present (6). These are MBL, which is a serum protein, SP-A, and SP-D. SP-A and SP-D are mainly produced in the epithelial cells of the lungs, but SP-D is also found in epithelial cells and secretory glands in the gastrointestinal tract and in other tissues

(7). SP-D plays an important role in the innate immune defense by binding to specific carbohydrate and lipid structures on the surface of microorganisms: bacteria, viral particles, fungi, and protozoa (8–11). This binding mediates effector mechanisms like aggregation, chemotaxis, mediation of phagocytosis, and permeabilization (12). Pulmonary infections in adults have been shown to cause significant changes in SP-D levels (13). Whether SP-D has a role in avoiding infections in newborn babies has to our knowledge not been investigated.

Both structural and promoter variants are known for the MBL gene, and several alleles correlate with low values of MBL in serum and lead to an increased risk of infectious diseases in otherwise healthy children (14,15). Three polymorphisms have been identified in the coding sequence of human SP-D: codons corresponding to amino acid residue 11 (Met11Thr), residue 160 (Ala160Thr), and residue 270 (Ser270Thr) in the mature protein. Two clinical studies have associated the SP-D variants of amino acid 11 with disease. The SP-D allele coding for methionine 11 has been associated with severe respiratory syncytial virus infection in infants, whereas threonine 11 has been suggested to increase susceptibility to tuberculosis (16,17). However, the connection between structural genetic variance and serum SP-D has not been investigated. We have performed a twin study comparing MBL

Received November 8, 2004; accepted March 9, 2005.

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Supported by grants from Direktør Ib Henriksens Foundation, Dagmar Marshalls Foundation, Gudrun Krauses Mindelegat, Overlægerådets legatudvalg, and Fonden for lægevidenskabelig forskning at Odense University Hospital

DOI: 10.1203/01.PDR.0000181379.72900.EC

and SP-D levels in 26 monozygotic and 36 dizygotic twins aged 6–9 y (18). The study showed a significant genetic influence on the serum levels of both MBL and SP-D, with a heritability level of approximately 90%.

Studies of amniotic fluid and lung tissue demonstrate increasing levels of SP-D with increasing gestational age (19–21). Levels of SP-A and D in amniotic fluid have therefore been suggested as markers of lung maturation (22,23). The levels of SP-A in umbilical cord blood also depend on gestational age and perinatal condition (24). However, the influence of gestational age and perinatal factors on SP-D levels has not been investigated previously.

The purpose of this study was to establish a normal range of SP-D in the umbilical cord blood and capillary blood of mature newborn infants. Furthermore, we wished to investigate the influence of perinatal factors on SP-D levels in mature newborn infants.

METHODS

Subjects. Out of a total of approximately 3600 newborn infants born at Odense University Hospital from August 2000 to August 2001, 458 (13%) were entered into the present study. Informed consent was obtained from the parents before birth by the midwives. Infants born before 36 weeks of gestational age or infants with major anomalies were excluded. Umbilical cord blood was drawn immediately after birth. The midwives were instructed to draw blood preferably from the umbilical artery. Routinely, all newborns are seen at the local midwife center at the age of 4–10 d. Within the project, infants attending the largest midwife center in the county additionally had blood taken for SP-D measurement. The capillary blood was sampled from heel-prick. All samples were stored at -20°C until measurement of SP-D.

Methodology. The SP-D concentrations in plasma were measured by enzyme-linked immunosorbent assay. The assay is based on pepsin-digested polyclonal rabbit anti-SP-D antibody as first-layer antibody, and monoclonal anti-SP-D antibody as detector antibody, as described earlier (13). The samples were tested in duplicates and accepted with a coefficient of variation of 5%.

Statistics. Data were analyzed by use of the STATA program. The main purpose of the study was to determine normal values for SP-D levels in the umbilical cord blood and capillary blood of newborns, so the median values and variance were not known before the study. Our chosen sample size was therefore limited to the number of samples we could obtain during the time period of the study.

The Mann-Whitney *U* test was used to compare levels of SP-D between two groups and the nonparametric test for trend across ordered groups for more than two groups. The variance between SP-D levels in arterial, venous, and capillary blood was tested by use of Bland-Altman's plot with Pitman's test of difference in variance. Correlations between continuous variables and SP-D levels were analyzed by simple regression analysis. In all statistical methods, $p \leq 0.05$ was considered significant. The results are expressed as medians (range) unless otherwise noted.

Ethical considerations. Written informed consent was given for every child enrolled in the study. The study was conducted according to the Helsinki II recommendations and was approved by the Regional Committee for Research on Human Subjects in the Counties of Funen and Vejle.

RESULTS

A total of 458 infants were included in the study. Umbilical cord blood was drawn from 423 infants, and the median SP-D plasma concentration was 392.1 ng/mL (range <20–1544.7). Of the umbilical blood samples, 130 were from the artery, with median SP-D level of 359.0 ng/mL (range 88.3–1505.0). Venous umbilical blood sampling was performed in 263 cases, and the median SP-D level was significantly higher than in the arterial samples: 420.8 ng/mL (range <20–1544.7, $p = 0.0013$). For statistical purposes, the term “umbilical cord

blood” was used for both arterial and venous samples, including 43 samples in which the label “arterial” or “venous” was missing. In 13 cases wherein both arterial and venous sampling was done, the term “umbilical cord blood” refers to arterial blood.

Capillary blood was sampled from 233 infants. The median SP-D level in capillary blood was 777.5 ng/mL (range 195.5–2669.1). This was significantly higher than in the umbilical cord blood ($p < 0.00005$). There was a significant correlation between the SP-D levels in umbilical cord blood and those in capillary blood (Bland-Altman, $p < 0.0005$). This correlation was found for both the arterial and the venous samples.

The infants' median gestational age was 40.3 weeks (range 36.0–43.4), and the median birth weight was 3600 g (range 1904–5164). We found significantly higher levels of SP-D in umbilical cord blood in the infants with lower gestational age (Fig. 1) ($p < 0.005$). However, regression analysis showed that the gestational age accounted for only 6.5% of the variation ($p < 0.0005$). There were no differences between different groups of gestational age with regard to the capillary SP-D levels. There was no correlation between birth weight and levels of SP-D in umbilical cord blood. The levels of SP-D in capillary blood decreased with increasing weight ($p = 0.05$).

Sixty-nine (15.6%) of the mothers had been smokers during pregnancy. The SP-D levels in umbilical cord blood (Table 1) of their infants were significantly lower than in infants of nonsmoking mothers ($p = 0.025$). This difference was found only in venous cord blood ($p = 0.0078$) and not in arterial cord blood ($p = 0.989$) (Table 2). The SP-D levels in umbilical cord blood (Table 1) were lower in infants born after more than 1 h of labor ($p = 0.039$). Ninety (19.7%) of the infants were delivered by cesarean section. The SP-D levels in umbilical cord blood did not differ according to mode of delivery, whereas the capillary SP-D (Table 3) was significantly higher in children delivered by cesarean section than in children delivered vaginally ($p = 0.002$). The infants delivered by

Figure 1. SP-D levels in umbilical cord blood ($n = 423$) and capillary blood ($n = 233$) at different gestational ages. SP-D concentrations in umbilical cord blood were significantly lower ($p < 0.005$) in groups with higher gestational age. Bars indicate median (95% CI) SP-D concentration. ■ Umbilical cord blood; □ Capillary blood.

Table 1. SP-D levels in cord blood in relation to maternal, obstetric, and neonatal conditions

Variable	Yes/no (n)	SP-D ng/mL		p
		Yes	No	
Maternal smoking	69/374	345.0 (318.3–400.5)	397.8 (378.9–427.9)	0.0251*
Maternal steroid	7/450	781.8 (295.3–1327.4)	388.1 (374.1–420.7)	0.0562
Labor	421/37	386.3 (369.3–416.6)	474.5 (374.6–653.7)	0.0390*
ROM	343/115	383.7 (365.1–412.3)	422.4 (382.0–465.7)	0.1010
CS	90/368	370.6 (341.1–421.1)	399.4 (377.9–428.3)	0.2407
CS $\pm$ ROM	54/36	341.1 (217.7–397.3)	441.3 (368.0–397.3)	0.0020***
Male	248/210	401.6 (377.6–439.0)	384.4 (358.7–426.4)	0.4297
IUGR	25/423	319.6 (265.9–481.5)	395.6 (378.3–426.0)	0.1895
Respiratory distress	14/444	365.1 (211.0–641.2)	392.4 (377.5–422.9)	0.7396
Antibiotics to child	9/449	385.7 (156.9–582.5)	392.3 (375.7–423.9)	0.7284

SP-D levels as median (lower and upper limit of 95% confidence interval).

ROM, rupture of membrane for >1 hour before birth; CS, cesarean section; IUGR, intrauterine growth retardation.

Values are compared by Mann-Whitney *U* test * = $p < 0.05$, *** = $p < 0.005$.

Table 2. SP-D levels in arterial and venous cord blood in relation to maternal smoking

Maternal smoking	Yes/no (n)	SP-D ng/mL		p
		Yes	No	
Arterial	108/19	345.0 (231.0–469.6)	359.0 (323.0–385.2)	0.9892
Venous	209/44	348.5 (293.5–420.7)	433.2 (394.4–474.0)	0.0078**

SP-D levels as median (lower and upper limit of 95% confidence interval).

Values are compared by Mann-Whitney *U* test, ** = $p < 0.01$.

Table 3. SP-D levels in capillary blood in relation to maternal, obstetric, and neonatal conditions

Variable	Yes/no (n)	SP-D ng/ml		p
		Yes	No	
Maternal smoking	26/198	635.9 (519.2–840.7)	784.6 (744.6–831.4)	0.0914
Maternal steroid	3/230	1061.8 (984.0–1533.8)	768.6 (738.4–818.1)	ND
Labor	222/11	768.6 (739.1–817.8)	1124.6 (593.2–1411.7)	0.1438
ROM	174/59	775.9 (739.5–831.8)	777.5 (631.9–902.4)	0.4354
CS	39/194	965.5 (835.7–1170.9)	754.6 (697.0–803.5)	0.0020***
CS $\pm$ ROM	27/12	867.2 (703.1–1068.9)	1152.0 (1057.4–1330.2)	0.1441
Male	125/108	794.4 (747.5–848.9)	763.4 (664.7–821.8)	0.1916
IUGR	12/221	1056.5 (696.2–1387.4)	766.8 (729.7–812.0)	0.0610
Respiratory distress	4/229	1518.8 (1195.5–1943.2)	767.5 (737.2–816.8)	ND
Antibiotics to child	6/227	1282.7 (671.5–1684.4)	766.8 (737.4–816.5)	ND

SP-D levels as median (lower and upper limit of 95% confidence interval).

ROM, rupture of membrane for >1 hour before birth; CS, cesarean section; IUGR, intrauterine growth retardation; ND, not done.

Values are compared by Mann-Whitney *U* test, *** = $p < 0.005$.

cesarean section with rupture of membranes for more than 1 h had significantly lower levels of SP-D in the umbilical cord blood ($p = 0.002$).

Seven (1.5%) of the mothers had been treated with beta-methasone on suspicion of premature labor 16–45 d before delivery. Maternal steroid treatment gave a tendency toward higher levels of SP-D in umbilical cord blood ($p = 0.056$) and in capillary blood. Only 4 infants had respiratory distress, and only 6 infants were treated with antibiotics on suspicion of septicemia within the first week of life. Their median SP-D level in capillary blood was almost twice as high as that of infants without respiratory distress or treatment with antibiotics, but the number was too small for statistical evaluation. None of the infants had proven septicemia.

The median age of the mothers was 29.9 y (range 18–44) at the time of delivery. The SP-D level in umbilical cord blood was lowest in the children of the youngest mothers ($p = 0.04$).

The capillary blood sampling was done at a median age of 6 d (range 0–11), and the SP-D level (Fig. 2) fell significantly from the time of birth ($p < 0.0005$).

DISCUSSION

We have in this study, to our knowledge for the first time, determined the SPD levels in umbilical cord blood and capillary blood from newborn infants. We found that the SP-D levels were highly variable, consistent with findings in healthy adult volunteers, and dependent on several maternal and perinatal conditions (13). The umbilical blood levels were found to depend on the mother's age and smoking habits, gestational age, and length of labor. Even though the study population was large (458 infants), a drawback of the study was the rather low inclusion rate of participation. However, the included infants did not differ from the whole population of mature newborn

Figure 2. Capillary SP-D in relation to age at blood sampling ($n = 233$). Concentrations of SP-D in capillary blood decreased significantly ($p < 0.0005$) with increasing postnatal age.

infants born during the study period with regard to gestational age, sex, and rate of cesarean section.

The capillary blood levels of SP-D were found to depend on the infants' birth weight, mode of delivery, and age at time of blood sampling. We found higher levels of SP-D in venous than in arterial umbilical cord blood, probably owing to the production of SP-D in the amniotic epithelium and choriodecidual layers (19,21). Increasing SP-D has been demonstrated in amniotic fluid with increasing gestational age (21,23). However, in these infants ≥ 36 wk of gestational age, we found decreasing amounts of SP-D in umbilical cord blood with increasing gestational age. This result probably reflects the decreasing amount of amniotic fluid in the fetal lungs toward term and perhaps a concomitant decrease in placental function resulting in decreasing SP-D production (25).

We found lower SP-D concentrations in umbilical cord blood of infants born after more than 1 h of labor. Uterine contractions have been reported to accelerate the secretion of pulmonary surfactant into the alveolar space and to decrease the amount of amniotic fluid by reabsorption of liquid across the pulmonary epithelium and outflow of liquid through the trachea (25–29). Three quarters of rat SP-D appears to exist in a soluble form, whereas 99% of rat SP-A exists in the form of a lipid protein complex (30). These findings may be related to the results of a clinical study of SP-A that showed elevated levels of SP-A with labor (24). These differences in SP-A and SP-D levels during labor could lead to the assumption that more SP-A is reabsorbed to plasma and more SP-D is transported by the outflow of liquid through the trachea. Furthermore, this assumption may also explain the decrease in SP-D observed in infants born by cesarean section more than 1 h after the rupture of membranes.

SP-D levels in infants with rupture of membranes for more than 1 h were not significantly lower than levels in infants without rupture of membranes before birth ($p = 0.10$), but a majority of the women had rupture of membranes for only a few hours (median 3 h). A study with a larger number of

women with rupture of membranes for more than 24 h would be interesting because one would expect SP-D in umbilical cord blood to decrease further.

We found a trend ($p = 0.056$) toward increased levels of SP-D in umbilical cord blood of mothers treated with steroids, which was consistent with experimental data showing increased SP-D expression after glucocorticoid treatment both *in vivo* and in cultured tissue (31,32). The levels of SP-D in the lungs of newborn infants have been found to rise significantly during the first days of life (33,34). This is possibly the explanation for the high levels in capillary blood compared with umbilical cord blood.

Infection and asphyxia in neonates with respiratory distress syndrome are thought to worsen an already impaired surfactant system (35). Injury to the alveolar epithelial barrier, as seen in acute respiratory distress, will lead to increased permeability, allowing leakage of large amounts of plasma proteins into the alveolar space, and a considerable amount of alveolar surfactant components will permeate the circulation (36,37). SP-D may, because of its more hydrophilic nature, enter the vascular compartment more easily than SP-A and has therefore been suggested as a valuable plasma biomarker of lung injury (37,38). Consistently with these findings, we observed high capillary levels of SP-D in infants with respiratory distress or suspicion of infection. Mechanical ventilation of the lungs has been shown to increase SP-D production (39). Probably the pressure delivered to the lungs by the nasal continuous positive airway pressure used to treat respiratory distress has the same effect (40). The number of sick infants was limited by the scope of our present study, but an ongoing study of SP-D levels in prematurely born infants is expected to elucidate the usefulness of SP-D as a biomarker of respiratory distress syndrome and infection.

Morbidity caused by lack of SP-D has not been described in humans. In our study we found one single infant with an SP-D level < 20 ng/mL. At the age of 1 year he was healthy, without any hospital visits. SP-D knockout mice show accumulation of surfactant lipids in the alveolar space, leading to emphysematous changes and increased susceptibility to infection (41,42). More clinical studies are needed to elucidate the effect in humans of low SP-D or absence of SP-D on pulmonary function and risk of infection.

Smoking during pregnancy decreases trophoblast proliferation and reduces the length of villous capillaries in the placenta, leading to diminished area for gas and nutrients exchange (43,44). Recently we have shown that SP-D is synthesized in all villous and extravillous trophoblast subpopulations in the placenta (19). In the present study, we found reduced SP-D levels in blood from the umbilical vein in smokers. This observation could have been due to reduced production or diminished transfer into the bloodstream. One could speculate whether the susceptibility of lung infections in infants of smoking mothers could be explained by these lower SP-D levels. However, the lower levels were found only in the umbilical venous blood and not in the umbilical arterial blood, which suggests that only the placental and not the fetal production of SP-D was affected.

In summary, we have in this study determined normal levels of SP-D in the blood of newborn infants born at term. Perinatal events may influence SP-D levels in opposing directions. Further studies with comparison of SP-D levels in the blood and in the mucosal tissues will be particularly interesting. Furthermore, the determination of SP-D levels in preterm infants, with their even more vulnerable innate as well as adaptive immune systems, may show more clearly the effects of variations in SP-D levels, as has been shown in experimental animals (45).

Acknowledgment. The authors thank Ivan Iachine for statistical assistance.

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