N-Terminal Propeptide of Type III Collagen in Tracheal Fluid and Serum in Preterm Infants at Risk for Bronchopulmonary Dysplasia

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ABSTRACT. Bronchopulmonary dysplasia (BPD) is a common pulmonary complication in preterm infants that leads to fibrosis of the bronchoalveolar walls and often to severe clinical consequences. Type III collagen is deposited early in progressive fibrosis. Because the N-terminal propeptide of type III collagen (PIIINP), a by-product of type III collagen synthesis, reflects the degree of pulmonary fibrosis in adults, we hypothesized that PIIINP in tracheal aspirates and/or serum may be a useful early marker of developing BPD in neonates. We serially measured PIIINP in tracheal fluid and serum samples during the first weeks of life in 41 consecutive respirator-treated preterm infants (mean birth weight 1067 g, mean gestational age 28.3 wk). Eight of the infants died and 22 infants fulfilled the criteria for BPD at age 28 d. The mean level of PIIINP decreased with advancing postnatal age in tracheal fluid but not in serum. The mean tracheal fluid PIIINP during d 1 and 2 of life, respectively, was 175 and 200 ng/mg protein in infants who were still in a respirator at age 28 d ($n = 13$), 122 and 97 ng/mg protein in those who were weaned earlier ($n = 20$), and 50 and 30 ng/mg protein in those who died before age 28 d ($n = 8$). These differences are not statistically significant, and the variability of the values was large. The PIIINP concentrations in tracheal aspirates of infants subsequently developing BPD did not differ from those without BPD. Neither did the levels correlate with the degree of BPD or radiologically defined fibrosis. In contrast to the findings in tracheal fluid, the serum PIIINP levels in infants subsequently developing BPD were significantly higher ($p = 0.01$) than those in infants without BPD, the respective mean (90% confidence intervals) concentrations being 788 (range 519–1057) and 256 (range 171–341) ng/mL. We conclude that serum PIIINP should be further evaluated as an early marker of BPD, whereas tracheal fluid levels are useless for this purpose. (*Pediatr Res* 31: 340–344, 1992)

Abbreviations

PIIINP, N-terminal propeptide of type III collagen
BPD, bronchopulmonary dysplasia
RDS, respiratory distress syndrome

BPD is a common complication of respirator treatment of pulmonary disease in the preterm infant. Its main risk factors

are immaturity, high inspired oxygen level, and barotrauma due to positive pressure ventilation (1). The incidence of BPD is roughly inversely proportional to gestational age.

The pathology of BPD develops from the typical findings of acute hyaline membrane disease through thickening and fibrosis of the bronchoalveolar walls (2, 3). Inflammatory cells and their proteolytic enzymes seem to contribute to tissue damage (4). In other types of organ fibrosis, connective tissue deposition begins with the synthesis of type III collagen, which is replaced by type I collagen at the later stages of disease (5–7). Consistent with this progression is the finding that the serum PIIINP, a by-product of type III collagen biosynthesis, is a sensitive early marker of developing liver and pulmonary fibrosis in adults (8, 9).

From the point of view of modifying treatment and estimating prognosis, identification of high risk of BPD or incipient disease in an individual infant would be desirable. Risk factors or scoring do not function well for this purpose (10), and methods to predict BPD by cytologic studies of tracheal aspirates have been unsatisfactory (11). Our hypothesis was that the PIIINP would appear in tracheal secretions and possibly in serum at the time when interstitial fibrosis in the lung starts to develop. To test this hypothesis, we performed a prospective clinical study on respirator-treated preterm infants.

MATERIALS AND METHODS

Patients. The study included 41 consecutive preterm infants with a birth weight of less than 1750 g who were intubated at birth, developed RDS, and required treatment in a respirator. Their clinical characteristics are shown in Table 1. RDS was defined clinically and radiologically as described (12), and the diagnosis of BPD was based on radiographic findings, need of extra oxygen, and clinical symptoms at age 28 d (13). None of the infants received surfactant treatment. Three infants had proven bacterial sepsis, and 22 were suspected of sepsis on the basis of clinical and laboratory data. An open ductus arteriosus required indomethacin treatment or surgery in 26 infants. Eight infants died at age 5 to 16 d (mean 10 d), seven of them with RDS, five of them with infection (septicemia, pneumonia, necrotizing enterocolititis, or tracheobrochitis), two of them with grade IV intraventricular hemorrhage, and one of them with pulmonary hemorrhage.

Data concerning respirator treatment and respiratory status were recorded and included respirator settings, blood gases, and clinical complications such as pneumothorax, pulmonary hemorrhage, and atelectasis. The pulmonary problems in the patients are shown in Table 2.

This study was carried out in the Children's Hospital, University of Helsinki, and it was approved by the ethical committee. Written consent was obtained from the parents of the infants.

Radiographic examinations. Anteroposterior supine chest

Table 1. *Clinical characteristics of 41 infants studied*

	Mean	Range
Gestational age (wk)	28.3	24–34
Birth wt (g)	1067	490–1620
Apgar score at 1 min	4.3	1–9
Apgar score at 5 min	6.6	1–10

Table 2. *Pulmonary problems in 41 respirator-treated infants*

	Yes (n)	No (n)	Dead (n)
Respirator treatment			
At 24 h	39	2	0
At 7 d	34	16	1
At 28 d	13	20	8
Pneumothorax	9	32	
Pulmonary hemorrhage	1	40	
Roentgenologic BPD at 28 d*	22	10	8
Infants fulfilling criteria for BPD	22	10	8
Roentgenologic fibrosis within first 28 d*	25	15	

* Roentgenograms of one patient were not available for analysis; fibrosis seen as interstitial prominence.

roentgenograms were read and scored by a pediatric radiologist (E.M.) on d 3, 7, 10, and 14 and thereafter at 1-wk intervals. The severity of RDS was scored on a scale from 0 to 5 as suggested by Edwards *et al.* (12). Radiographic changes related to BPD were scored from 0 to 10 on the basis of fibrosis, interstitial air trapping, cardiovascular changes, hyperexpansion of the thorax, and the general impression of the roentgenograms (13). If the technical quality of chest radiograph was suboptimal, another taken on the previous or following day was used.

Samples. A total of 267 tracheal aspirates (an average of seven samples from each patient, range two to 11) were collected into DeLee traps at the time of routine suctioning of intubation tubes daily during the first week of life and weekly thereafter for the duration of respirator treatment. The samples (volume in the order of 0.2–1.0 mL) were mixed with protease inhibitor PMSF and NEM (phenylmethylsulfonyl fluoride and N-ethylmaleimide) to the final concentrations of 25 mM EDTA, 1 mM para-aminobenzoic acid, 1 mM PMSF, and 10 mM NEM and kept at −20°C until analyzed. A total of 63 serum samples from a subset of 32 infants were collected weekly at the time of routine laboratory tests. In addition, cord blood samples from 69 infants between 24 and 42 gestational wk, chosen at random from routine screening for congenital hypothyroidism (14), were studied to evaluate the ontogenesis of serum PIIINP concentrations.

Biochemical methods. The PIIINP concentrations in tracheal and serum samples were measured with a specific RIA (Hoechst AG, Frankfurt Am Main, Germany) (8) with an intraassay coefficient of variation of 4.8%. The PIIINP content was measured in each individual sample, and no pooled samples were used. To correct for variable dilution of the tracheal aspirates, the results are expressed on the basis of protein concentration (as ng of PIIINP/mg protein) measured by the commercial protein assay (Bio-Rad Laboratories, Richmond, CA).

Statistical methods. Because of the large variation in the tracheal samples, means and 90% confidence intervals are given and nonparametric statistics (the Mann-Whitney test) were used for comparing prognostic groups. Cord serum PIIINP was related to gestational age using regression analysis, and postnatal serum values were compared using the Mann-Whitney test.

RESULTS

PIIINP in tracheal aspirates. The mean levels of PIIINP in tracheal fluid declined during the first week of life and more profoundly after age 3 wk (Fig. 1). The concentration of PIIINP in the aspirates was not related to the age of onset, clinical stage,
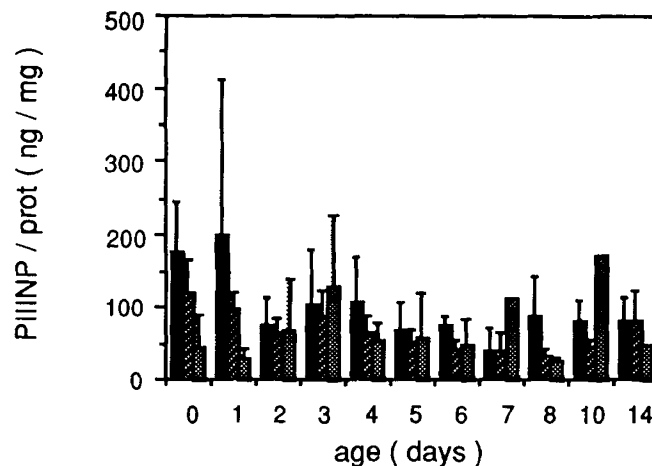

Fig. 1. Tracheal fluid PIIINP concentrations (ng/mg protein) (mean and the upper 90% confidence limit) during the first 2 wk of life in various prognostic groups: infants who were still in a respirator at d 28 (*solid bars*), infants who were weaned before d 28 (*diagonally striped bars*), and infants who died before d 28 (*shaded bars*).
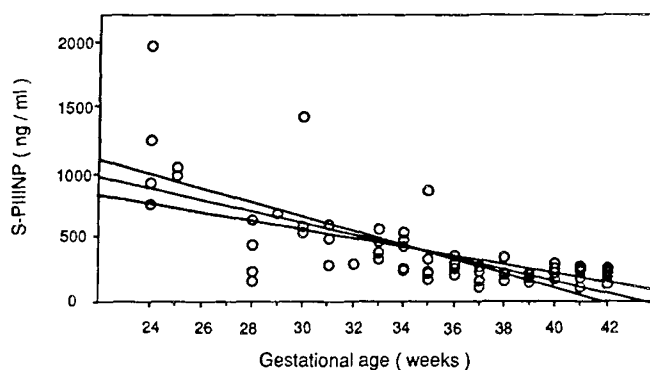

Fig. 2. PIIINP concentrations (ng/mL) in cord blood serum as a function of gestational age. The lines indicate 95% confidence limits.

or radiologic grade of RDS in the premature infants. Neither did the PIIINP concentration, measured at any time, correlate with the time of appearance of the first radiologic signs of BPD, the degree of radiologically defined fibrosis, or total BPD score. The mean (± SD) radiologic BPD score at 28 d in infants with BPD was 6.6 (± 2.2) (scale 1–10). The infants fulfilling the criteria for BPD did not differ from the other infants with respect to their PIIINP levels. The mean (90% confidence limits) concentration of PIIINP at age 1 and 2 d was 175 (105–245) and 200 (0–412) ng/mg protein, respectively, in infants who were still in a respirator at age 28 d ($n = 13$), 122 (76–167) and 97 (73–121) ng/mg protein in those who were weaned earlier ($n = 20$), and 50 (7.8–92) and 30 (20–41) ng/mg protein in those who died before age 28 d ($n = 8$), but the differences were not statistically significant (Fig. 1). The corresponding protein concentrations (90% confidence intervals) in the aspirates from the three groups were 0.85 (0.26–1.45), 0.81 (0.30–1.32), and 2.21 (0–7.3) mg/mL on d 1 and 0.19 (0.12–0.27), 0.33 (0.18–0.48), and 0.42 (0.31–0.81) mg/ mL on d 2, respectively. Of these protein levels, the difference was statistically significant on d 2 between the respirator group and the spontaneously breathing group ($p = 0.0001$) and between the respirator group and the group of infants who died before age 28 d ($p = 0.0001$). Such differences in the PIIINP or protein content were not seen on d 3 or later.

Other pulmonary problems or infections did not influence the PIIINP levels in tracheal fluid of these infants. No correlation with any respiratory parameter was detected.

PIIINP in serum. The PIIINP concentration in cord blood declined with advancing gestational age (Fig. 2, $r = 0.69$, $p =$

0.0001). However, in the postnatal samples, serum PIIINP levels showed no correlation with the postconceptional or postnatal age. Therefore, all samples of each prognostic group taken before age 28 d were analyzed together. The mean (90% confidence interval) level during d 1 through 22 (mean 5 d) of life in infants subsequently developing BPD (32 samples) was 800 (498–1102) ng/mL, and in infants without BPD (22 samples) the mean level was 256 (171–341) ng/mL ($p = 0.002$, Fig. 3). The mean (90% confidence interval) concentrations were highest [794 (376–1213) ng/mL] in infants who required respirator treatment beyond d 28. Those infants who were weaned earlier had a mean level of 451 (271–631) ng/mL, and infants who died before d 28 had a mean level of 284 (149–418) ng/mL (Fig. 3). These differences were not statistically significant.

DISCUSSION

Various biochemical markers have been studied in infants with RDS and subsequent BPD. Tracheal fluid fibronectin has been reported to be elevated in premature infants with BPD as compared with infants with RDS or without respiratory difficulties (15). Proteolytic injury to the lung tissue in BPD is reflected in lower serum concentrations of α-1-proteinase inhibitor, the major modulator of elastase activity. Elastase activity in tracheal aspirates, on the other hand, has been shown to be raised in infants with BPD (4). There is, however, no good biochemical or cytologic marker available for the early detection of preterm infants at risk for developing BPD. Our hypothesis was that PIIINP, reflecting the synthesis of type III collagen, would be such a marker. In adults, PIIINP levels have been found to be elevated in alveolar fluid in patients with idiopathic pulmonary fibrosis (16) and in serum and bronchoalveolar lavage fluid in patients with adult RDS (17) known to be complicated with severe fibrosis. In infants with advanced chronic lung disease, the ratio of type I/III collagen has been reported to be elevated, mainly because of elevated amounts of type I collagen in the lungs (18). The levels of type I or III collagen in ventilated infants have not, however, been studied during the development of lung damage. The PIIINP serum levels have also been shown to be an early indicator of liver fibrosis in patients with alcoholic liver disease and chronic active hepatitis (8, 19).

Contrary to our expectations, the PIIINP concentrations in 1st-wk tracheal aspirates of infants developing BPD did not differ from those without BPD. Neither did they correlate with the degree of BPD nor of radiologically defined fibrosis. Although infants with a long respirator treatment seemed to have higher tracheal aspirate PIIINP levels than those who were weaned earlier, the difference was not significant. This may be due to the large variation in volume, protein, and PIIINP concentration in tracheal samples, which is partly explained by the variable production of bronchoalveolar secretion and its variable dilution with saline used for flushing.

The tracheal fluid PIIINP levels were lowest in infants who died because of respiratory failure. This could be partly due to the fact that the most severe RDS is known to be associated with the most extensive protein leak to the alveolar fluid (20). This was also the case in our study: those infants who died early had the highest protein content in the aspirates, which partly accounts for the low PIIINP value per mg protein in this group of patients. Protein concentration is thus not an optimal reference basis for expressing the results. Tracheal fluid albumin and urea were not studied in our work, but these could possibly serve as alternative references bases to express the PIIINP results. The difference in tracheal fluid PIIINP concentration was not evident on d 3 or later, when there were no more differences in the protein concentrations of the tracheal aspirates. On the other hand, the lowest serum PIIINP concentrations were also noted in those infants with the worst prognosis, suggesting that factors other than the protein content may contribute to the differences in tracheal aspirates as well.

The serum PIIINP concentration in healthy children is high at birth and declines during childhood before it again increases at puberty (21, 22). The fetal serum levels or the effect of gestational age on serum PIIINP concentrations have not been studied previously. The analysis of cord blood sera showed a clear decline in PIIINP concentrations with advancing gestation. However, the serum levels in preterm infants were not correlated with postnatal or postconceptional age, suggesting that factors other than maturity have a dominant influence. The differences between the prognostic groups suggest that lung damage may be such a factor.

Our finding of somewhat higher serum levels of PIIINP in infants subsequently developing BPD, as compared with infants without BPD, encourages further studies on the possible use of this marker for monitoring preterm infants at risk for BPD. Tracheal samples appear useless for this purpose.
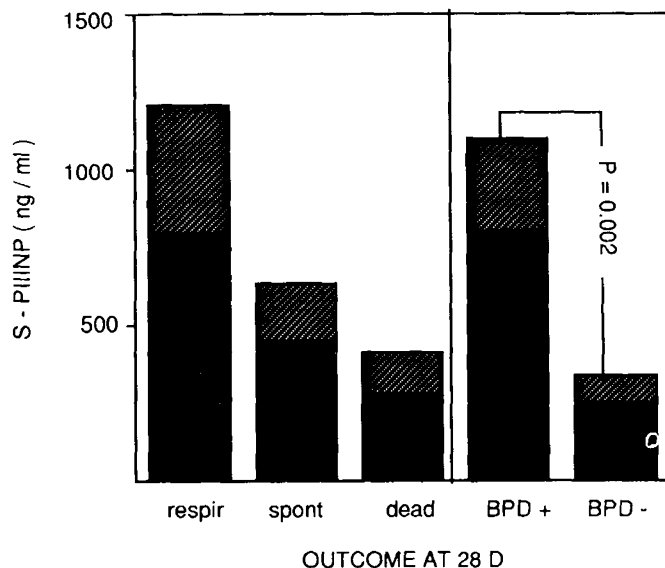

Fig. 3. Serum PIIINP concentrations (ng/mL) (mean and the upper 90% confidence limit) in different prognostic groups according to the length of respirator treatment (*respir*, at respirator at age 28 d; *spont*, spontaneously breathing at age 28 d; *dead*, dead before age 28 d) and in infants with BPD (*BPD+*) and without BPD (*BPD−*).

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