Airway Concentrations of Angiopoietin-1 and Endostatin in Ventilated Extremely Premature Infants Are Decreased After Funisitis and Unbalanced With Bronchopulmonary Dysplasia/Death

WOLFGANG THOMAS, SILVIA SEIDENSPINNER, BORIS W. KRAMER, NATALIA KAWCZYŃSKA-LEDA, MARIA CHMIELNICKA-KOPACZYK, ALEXANDER MARX, JOHANNES WIRBELAUER, MARTA SZYMANKIEWICZ, AND CHRISTIAN P. SPEER

University Children's Hospital [W.T., S.S., J.W., C.P.S.], University Hospital Würzburg, Würzburg 97080, Germany; Department of Pediatrics [B.W.K.], Maastricht University Medical Center, Maastricht 6202 AZ, The Netherlands; Departments of Neonatology [N.K.-L., M.S.] and [M.C.-K.], Poznań University of Medical Sciences, Poznań 60-535, Poland; Institute of Pathology [A.M.], University of Heidelberg, Mannheim 68167, Germany

ABSTRACT: A systemic inflammatory response of the fetus, reflected by histologic funisitis, is a risk factor for bronchopulmonary dysplasia (BPD). Impaired pulmonary angiogenesis accompanied by simplification and rarification of alveoli is a histologic hallmark of BPD. Angiopoietin-1 mediates vascular development, maturation, and stabilization. Endostatin mainly acts as an angiostatic factor. We hypothesized that funisitis was associated with changes of endostatin and angiopoietin-1 concentrations in the airways and that an imbalance between the factors might be associated with BPD or death. We measured concentrations of angiopoietin-1 and endostatin by enzyme-linked immunosorbent assay in tracheobronchial aspirate fluid samples of 42 ventilated preterm infants during postnatal days 1 through 15. The secretory component for IgA served as reference protein. A standardized histologic examination was used to distinguish three groups: chorioamnionitis, funisitis, and controls without inflammation. Concentrations of the mediators steadily decreased. Funisitis was associated with lower concentrations of both proteins, which might impair their physiologic activities in pulmonary angiogenesis. An increase of the ratio angiopoietin-1/endostatin until day 7 of life indicated a shift of the mediators potentially favoring angiogenesis. However, infants, who developed BPD or died, had a decreased ratio on days 1, 3, and 15, suggesting an imbalance toward inhibition of pulmonary angiogenesis. (*Pediatr Res* 65: 468–473, 2009)

In extremely premature infants, pre- and postnatal lung injury interferes with normal lung development potentially leading to bronchopulmonary dysplasia (BPD) (1). The typical histologic pattern of BPD is a combination of alveolar simplification and decreased capillary density (2). Evidence from animal models suggest that pulmonary angiogenesis is a prerequisite for normal alveolar development (3,4). Therefore, impaired pulmonary angiogenesis might not only accompany but also cause alveolar simplification in BPD (5). A potential mechanism

for disturbed angiogenesis is an imbalance between pro- and antiangiogenic factors triggered by inflammation (5–7).

The angiogenic mediator angiopoietin-1 is the primary agonist of the tyrosine kinase receptor Tie2. Angiopoietin-1 inhibits apoptosis, has chemotactic and mitogenic effects on endothelial cells, and supports the localization of adhesion molecules in endothelial intercellular junctions, thereby stabilizing blood vessels (8). Angiopoietin-1 promotes differentiation of mesenchymal cells in vascular smooth muscle cells and paracrine secretion of angiopoietin-1 as well as constitutive expression of Tie2 plays a pivotal role in maintaining the integrity of mature quiescent vasculature (8). Angiopoietin-1-Tie2 interaction inhibits nuclear factor kappa B (9) leading to a reduced transcription of proinflammatory mediators (10).

Endostatin is activated by proteolytic cleavage from its precursor collagen XVIII (11,12). It inhibits endothelial cell proliferation, migration, and tube formation. Gene profiling and protein phosphorylation studies revealed that endostatin is able to downregulate endothelial signaling cascades associated with proangiogenic activity (11,12). Simultaneously, it upregulates a multitude of antiangiogenic genes (11,12). In cytological studies, it has both anti- as well as proangiogenic effects, depending on dosages and endothelial cell types (13,14). Endostatin is consistently found in tracheobronchial aspirate fluid (TAF) of preterm infants in the first weeks of life and in the pulmonary endothelium at different stages of pre- and postnatal development (15), suggesting a role in physiologic lung development.

Funisitis, characterized by neutrophilic infiltration, associated with upregulation of vascular adhesion molecules in the vessels of the umbilical cord (16) and activation of endothelial inflammatory processes in the placental capillaries (17), reflects the most invasive form of prenatal inflammation with a systemic response of the fetus, that has been associated with higher neonatal mortality (18) and BPD (19).

Received August 5, 2008; accepted November 11, 2008.

Correspondence: Wolfgang Thomas, M.D., University Children's Hospital, University Hospital Würzburg, Josef-Schneider-Str. 2, Würzburg, Bavaria 97080, Germany; e-mail: Thomas_w@klinik.uni-wuerzburg.de

Parts of the data were presented at the 23rd German Congress on Perinatal Medicine, Berlin, Germany, November 2007, and the 23rd International Workshop on Surfactant Replacement, Bruges, Belgium, June 2008.

Abbreviations: BPD, bronchopulmonary dysplasia; IgA-SC, secretory component for IgA; TAF, tracheobronchial aspirate fluid

In our clinical prospective study, we explored the association between prenatal inflammation, concentrations of angiopoietin-1 and endostatin in TAF, the balance between the two factors and respiratory outcome or death in ventilated extremely premature infants. We speculated that a systemic fetal response, reflected by histologic funisitis, could be associated with changes of the factors' airway concentrations and that an imbalance between the factors could be associated with adverse respiratory outcome.

METHODS

Patients. Forty-two infants with a birth weight <1500 g and a gestational age <30 wk, who were consecutively admitted to the intensive care unit of the Department of Neonatology, Poznań University of Medical Sciences, Poland, were enrolled in this clinical prospective study after informed parental consent. All patients were inborns. The causes of preterm delivery were preterm premature rupture of membranes/amnion infection syndrome in 19, multiple gestations in 9, abruption of placenta in 5, pregnancy-induced hypertension in 6 cases, a malformation of the uterus in one case, and intrauterine growth restriction in another one. One infant had no overt risk factor. Infants were enrolled if they had to be mechanically ventilated for respiratory distress syndrome within the first 48 h of life. Patients with major congenital malformations were excluded. Conventional mechanical ventilation was used in all patients and adjusted to reach a SpO_2 between 85 and 94%. High frequency oscillation ventilation was used in four patients as rescue therapy. Antibiotic therapy with ampicillin and gentamicin was initiated in all patients. Thirty-six patients had received prenatal steroids. A single dose of hydrocortisone was administered to four infants on days 1, 3, 6, and 9 of life, respectively. Eleven patients received dexamethasone before discharge, but only two in the phase of life when we collected TAF (days 8 and 10, respectively). Twenty-six patients were treated with up to three doses of natural bovine surfactant for respiratory distress syndrome with a need for supplemental oxygen >40%.

Infants with supplemental oxygen for at least 28 d were assigned to have BPD (20). According to current definitions, severity of BPD was classified by the need for supplemental oxygen at the age of 36 postmenstrual weeks as mild (no supplemental oxygen), moderate (22–30% oxygen), or severe (>30% oxygen) (20). The study was approved by the ethical committees of the faculty of medicine in Würzburg and the Poznań University of Medical Sciences.

Histologic examinations. Two sections of umbilical cord, two sections of placental parenchyma, and one section of a membrane roll were evaluated for neutrophilic infiltrates by two pathologists, unaware of clinical data, according to a recently published protocol (21). Patients were assigned to three groups according to histologic diagnosis. The first group was characterized by chorioamnionitis and funisitis and will be referred to as funisitis group ($n = 17$). In the second group, chorioamnionitis without funisitis was diagnosed (chorioamnionitis group, $n = 9$). The control group consisted of 16 patients without inflammation in placenta, membranes, and umbilical cord.

Sampling, preparation, and storage of TAF. Tracheobronchial aspiration was performed in a standardized manner three times per day on days 1, 3, 5, 7, 10, and 15 of life. After instillation of 0.5 mL NaCl 0.9% into the endotracheal tube, a suction catheter was inserted slightly beyond the distal tip of the tube and TAF was collected in sterile mucus traps (Unomedical, Birkerød, Denmark). Each specimen was diluted with NaCl 0.9% to a volume of 0.5 mL. Specimens of 1 d were pooled. They were spun at 3000 rounds per minute (rpm) for 5 min. Supernatants were centrifuged again twice at 10,000 rpm for 5 min. The resulting clear supernatants of 1 day were divided into aliquots and stored at -80°C until further processing.

Angiopoietin-1, endostatin, and secretory component for IgA. Concentrations of angiopoietin-1 and endostatin were measured with commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). To avoid errors due to the sampling procedure, concentrations were related to the secretory component for IgA (IgA-SC). Levels of IgA-SC in TAF are independent of capillary leak and not affected by gestational or postnatal age during the first month of life (22). IgA-SC was measured by enzyme-linked immunosorbent assay as previously described (23). The number of patients in which TAF analysis for angiopoietin-1, endostatin, and IgA-SC could be performed were as follows: control group: day 1, 13 of 16; day 3, 16 of 16; day 5, 15 of 16; days 7 and 10, 14 of 16 and day 15, 11 of 16; chorioamnionitis group: day 1, 7 of 9; days 3–7, 9 of 9; day 10, 8 of 9; day 15, 5 of 9; funisitis group: days 1 and 3, 17 of 17; days 5 and 7, 14 of 17; day 10, 12 of 17 and day 15, 6 of 17.

Statistical analysis. Birth weight, gestational age, and umbilical cord arterial pH were compared between groups using one-way analysis of variance. Values are expressed as means $\pm$ SD. All other intergroup differences for continuous variables were assessed by Kruskal-Wallis test followed by Mann-Whitney U test for pairwise comparison. These data are expressed as medians (lower-upper quartile). Sequential values of angiopoietin-1, endostatin, and the ratio angiopoietin-1/endostatin were analyzed by Friedman test for the complete course and by Wilcoxon's rank sum test for pairs. Correction for multiple comparisons was done with Bonferroni's method. Categorical and nominal values were compared by χ^2 test. Correlations between continuous or ordinal variables were assessed by Spearman's rank correlation coefficient. All tests were two sided, a value of $p < 0.05$ was considered significant. Statistical evaluation was performed with SPSS 15 for Windows (Chicago, IL).

RESULTS

Patients. Clinical characteristics and outcomes of patients are summarized in Table 1. In the chorioamnionitis group, birth weights were lower than in the control and funisitis group ($p = 0.01$) but not the gestational age. The maximal blood leukocyte count in the first 3 days of life was higher ($p < 0.001$), preterm premature rupture of membranes was more prevalent, and the interval between rupture of membranes and birth was longer in the funisitis group compared with chorioamnionitis and control ($p < 0.001$). Administration of prenatal and postnatal corticosteroids or surfactant did not differ between groups. There was no difference in respiratory outcome measures such as duration of mechanical ventilation, incidence of BPD, or duration of oxygen therapy either. Ten infants died until day of life 25. They had high-grade intraventricular hemorrhage (4), overwhelming sepsis (5), or pulmonary hemorrhage (1). Mortality varied significantly between groups ($p = 0.02$).

Nineteen survivors developed BPD, 13 had mild, 6 moderate, and no infant severe BPD. Table 2 illustrates differences in demographic and clinical parameters between survivors with and without BPD and deaths. Infants who died had a lower gestational age ($p = 0.001$) and birth weight ($p = 0.005$) and were more likely to be males ($p = 0.025$). Survivors who developed BPD received more often postnatal steroids ($p = 0.024$) and were ventilated for a longer period ($p < 0.001$).

In 40 patients, airway secretions were cultured for *Ureaplasma urealyticum*. The prevalence of this microbe was 11 of 40 patients and did not differ between groups. There was no association between *U. urealyticum* and respiratory outcome.

Angiopoietin-1 and endostatin in TAF. Concentrations of angiopoietin-1 and endostatin in TAF decreased from day 1 of life to day 15 ($p = 0.02$ for angiopoietin-1, $p < 0.001$ for endostatin). The median angiopoietin-1/IgA-SC was 67.5 pg/ μg (28.9–122.7) on day 1 and 30.8 pg/ μg (9.3–48.3) on day 15 ($p < 0.05$), the median endostatin/IgA-SC 1280 pg/ μg (500–2770) on day 1 and 305 pg/ μg (160–533) on day 15 ($p < 0.01$).

Figure 1 illustrates the concentrations of both mediators in the groups control, chorioamnionitis, and funisitis. The funisitis group had significantly lower angiopoietin-1/IgA-SC than the control group on days 1–3 ($p < 0.001$) and lower values than the chorioamnionitis group on days 5–7 ($p = 0.013$ and 0.005 , respectively, panel A). For endostatin, the pattern was similar (panel B). In the funisitis group, endostatin/IgA-SC was significantly decreased vs. control on days 1–5 and 10 and vs. chorioamnionitis on days 1–10 ($p < 0.01$).

Table 1. Clinical characteristics and short-term outcome of the study population

	Control (n = 16)	Chorioamnionitis (n = 9)	Funisitis (n = 17)	p
Gestational age (wk)*	28.1 ± 1.2	26.5 ± 1.8	27.1 ± 2.1	0.06
Birth weight (g)*	1048 ± 186	827 ± 186	1088 ± 241	0.01
Male	5	6	7	0.23
Umbilical cord arterial pH*	7.25 ± 0.14	7.31 ± 0.10	7.31 ± 0.11	0.27
Apgar score 5 min†	7 (5–8)	7 (6–7)	6 (5.5–7)	0.39
Maximal leukocyte count day 1–3 (n/nL)†	13.35 (10.2–16.2)	10.8 (8.65–16.85)	28.9 (19.45–43.25)	<0.001
PROM >18 h	2	0	13	<0.001
Interval ROM—birth (h)†	0 (0–6)	0 (0–0)	71 (20–432)	<0.001
Prenatal steroids	13	8	15	0.43
Postnatal steroids				0.37
Hydrocortisone	0	2	2	
Dexamethasone	5	1	5	
Surfactant therapy	11	7	8	0.24
<i>Ureaplasma urealyticum</i> in TAF	2	2	7	0.21
Death	1	5	4	0.02
BPD in survivors				0.92
No	7	1	5	
Mild	5	2	6	
Moderate	3	1	2	
Severe	0	0	0	
Days on mechanical ventilation in survivors†	19 (14–35)	25 (18.5–30.75)	18 (11.5–29.5)	0.58

* Values are means ± SD.

† Values are medians (lower-upper quartile).

(P)ROM, (premature) rupture of membranes.

Table 2. Significantly different demographic and clinical characteristics according to respiratory outcome/death

	Survivors, no BPD (n = 13)	Survivors, BPD (n = 19)	Deaths (n = 10)	p
Gestational age (wk)*	28.3 ± 1.4	27.6 ± 1.5	25.7 ± 1.7	0.001
Birth weight (g)*	1155 ± 132	1005 ± 216	857 ± 256	0.005
Male	4	6	8	0.025
Postnatal steroids				0.024
Hydrocortisone	0	1	3	
Dexamethasone	3	8	0	
Days on mechanical ventilation in survivors†	12 (9.5–19)	28 (18–35)		<0.001

* Values are means ± SD.

† Values are medians (lower-upper quartile).

Surfactant therapy, postnatal corticosteroids, a hemodynamically relevant patent ductus arteriosus, and colonization with *U. urealyticum* were not associated with significant alterations of angiopoietin-1/IgA-SC or endostatin/IgA-SC in TAF (data not shown). Concentrations of the mediators were not associated with severity of BPD, death, or the combined outcomes moderate BPD/death and BPD/death (Fig. 2). Gestational age did not correlate with angiopoietin-1/IgA-SC or endostatin/IgA-SC at any time. This was the same for birth weight and angiopoietin-1/IgA-SC. However, birth weight correlated negatively with endostatin/IgA-SC on days 1 ($r = -0.33$), 3 ($r = -0.35$), 7 ($r = -0.36$), and 10 ($r = -0.43$); $p < 0.05$, respectively.

Ratio angiopoietin-1/endostatin. The ratio angiopoietin-1/endostatin for each individual patient and day was calculated to assess the balance between the angiogenic and angiostatic mediator in the infants' airway secretions. For the complete cohort, the ratio increased steadily from day 1 to 7 and then gradually declined (Fig. 3A). Values were significantly lower on day 1 than on days 5–10 ($p < 0.001$), and on day 3 lower than on day 7 ($p = 0.001$). Surfactant therapy, corticosteroids, PDA, and *U. urealyticum* were not associated with any

changes in the ratio. There was no significant difference between the groups according to prenatal inflammation with the exception of day 3 (Fig. 3B).

The course of the ratio showed comparable patterns for infants who survived without BPD, who developed BPD or who died. However, the ratio was significantly lower in those infants who developed BPD or died on days 1 ($p = 0.01$), 3 ($p = 0.002$), and 15 ($p = 0.045$) (Fig. 3C). Sixteen patients, who developed moderate BPD or died, had significantly lower values on day 3 ($p = 0.019$) and on day 15 ($p = 0.01$), compared with the remaining 26 infants. In survivors, values were still significantly lower in the BPD group on days 1 ($p = 0.037$) and 3 ($p = 0.009$) with a trend to lower values on day 15 ($p = 0.073$).

DISCUSSION

We found that concentrations of angiopoietin-1 and endostatin in TAF of ventilated extremely premature infants steadily decreased within the first 15 d of life in this clinical prospective study. In our cohort, histologic funisitis, reflecting a fetal inflammatory response syndrome (24,25), was associ-

Figure 1. Boxplots of angiopoietin-1/IgA-SC (A) and endostatin/IgA-SC (B) in TAF of the three groups control ($n = 16$, dark gray), chorioamnionitis ($n = 9$, gray), and funisitis ($n = 17$, white). The bold bars indicate the median, the boxes the interquartile range, and the error bars the 5th and 95th percentile. Circles indicate outliers and asterisks extreme values. In the funisitis group, angiopoietin-1/IgA-SC was significantly lower compared with the control group on days 1–3 ($p < 0.001$) and compared with the chorioamnionitis group on days 5–7 ($p = 0.013$ and 0.005 , respectively). In the funisitis group, endostatin/IgA-SC was significantly decreased vs. control on days 1–5 and 10 and vs. chorioamnionitis on days 1–10 ($p < 0.01$).

Figure 2. Angiopoietin-1/IgA-SC and endostatin/IgA-SC for the outcome BPD or death ($n = 29$, gray). There was no significant difference compared with concentrations of both mediators in TAF of the remaining 13 patients (dark gray).

ated with low concentrations of both mediators in TAF, compared with infants who had either experienced a localized inflammation of the placenta and membranes or none.

In vitro, the proinflammatory cytokine interleukin-1 β was shown to downregulate angiopoietin-1 mRNA expression in human lung fibroblasts (26) potentially leading to reduced concentrations on protein level like in our cohort after systemic fetal inflammation. In a baboon model of BPD, mechanical ventilation was not associated with changes in pulmonary

mRNA expression for angiopoietin-1 (27). However, angiopoietin-1 mRNA was downregulated in lung tissue of very preterm infants after mechanical ventilation as well as mRNA for vascular endothelial growth factor (VEGF) (28). These data correspond to our findings of steadily decreasing angiopoietin-1 protein concentrations in ventilated extremely preterm infants. Angiopoietin-1 stabilizes VEGF-induced pulmonary capillaries and contributes to enhanced alveolarization (29). Thus, one might speculate that low airway concentrations of the angiogenic factor after funisitis reflect a predisposition for abnormal pulmonary angiogenesis and abnormal alveolar development, which could be further aggravated by postnatal factors like mechanical ventilation.

Recently, Janer *et al.* (15) described a steady decrease of endostatin concentrations in TAF of very low birth weight infants over the first weeks of life. In Janer's (15) cohort, the clinical diagnosis of chorioamnionitis was associated with decreased concentrations of endostatin. As the clinical diagnosis of funisitis (25), a considerable number of Janer's "chorioamnionitis" patients would probably have qualified for our funisitis group. Likewise, preterm labor is often induced by clinically silent chorioamnionitis. In this respect, our findings of decreased concentrations of endostatin only with systemic fetal inflammation may specify Janer's (15) data. Janer *et al.* (15) suggested that lower endostatin concentrations may reflect accelerated lung maturation because they decreased with increasing postnatal age and they negatively correlated with birth weight and with initial lecithin/sphingomyelin ratios in TAF of their cohort. Fetal exposure to inflammation does induce lung maturation (30,31), but this maturational effect is accompanied by a predisposition to ongoing pulmonary inflammation induced by postnatal events (32), eventually leading to adverse pulmonary outcome (30). Besides, decreasing postnatal endostatin concentrations in TAF over time might also reflect the influence of iatrogenic interventions. Prolonged oxygen supplementation for example, was shown to reduce pulmonary endostatin mRNA levels in newborn piglets (33) and prenatal betamethasone was reported to be associated with higher TAF concentrations of endostatin in preterm infants (15).

Pulmonary angiogenesis is a complex process, which requires the activity of multiple molecules in a specific temporospatial order (34). A concentration-dependent modulating—rather than only inhibiting—effect of endostatin on embryonic vasculo- and angiogenesis has been demonstrated *in vitro* (13,14). The parallel suppression of angiopoietin-1 and endostatin in the funisitis group might reflect a failure to reach local pulmonary threshold concentrations of both factors necessary for a physiologic activity in lung vascular and alveolar development (13,14,34).

The role of many angiogenesis-modifying factors in human lung development has recently been assessed. Increasing pulmonary expression of endothelial nitric oxide synthase, endothelin-1, and VEGF over gestation with a peak at the gestational age of the infants in our cohort—corresponding to the transition from the saccular to the alveolar phase of lung development—underlines the importance of angiogenesis for

Figure 3. Ratio angiopoietin-1/endostatin. *Panel A:* For the complete cohort it increased until day 7 and then gradually declined. The ratio was significantly lower on day 1 than on days 5–10 ($p < 0.001$), and on day 3 lower than on day 7 ($p = 0.001$). *Panel B:* There was only a significant difference for the ratio according to severity of prenatal inflammation on day 3 ($p = 0.004$ for control vs. chorioamnionitis; control = dark gray, chorioamnionitis = gray, funisitis = white). *Panel C:* The ratio was significantly lower in those infants who developed BPD or died (gray) on days 1 ($p = 0.01$), 3 ($p = 0.002$), and 15 ($p = 0.045$).

the process of alveolarization in human lungs (35). However, results of studies focusing on the association between postnatal concentrations of these factors in airways of ventilated preterm infants and evolving pulmonary disease have been inconsistent (36–39). Respiratory distress and BPD were described to be associated with lower TAF concentrations of endothelin-1 (37) and VEGF (39) in the first days of life or with no changes of endothelin-1 (36) or VEGF (36,38) at all.

In our cohort, we did not find an association between funisitis and development of BPD (1,19) or mortality (18) and we did not find an association between the concentrations of angiopoietin-1 and endostatin in TAF and the outcomes BPD or BPD/death either. In both groups, the pattern of gradually decreasing concentrations of the mediators after birth was evident. However, many postnatal factors, which we could not control in our clinical study, contribute to the evolvement of BPD (40) or death (41).

Clinical data suggest that, besides absolute concentrations, the balance between modulators of angiogenesis plays a role in disease processes involving the lung (42). The balance between mediators is important for the process of controlling vascular growth and maturation (43). We used the ratio angiopoietin-1/endostatin in TAF to assess the balance between the two mediators. For the complete cohort, we demonstrated an increase of this ratio in the first week of life, suggesting a shift of the balance between the mediators toward angiogenesis. We cannot preclude that lower ratios could be at least partially due to an increase of endostatin after prenatal steroids as previously demonstrated (15). Prenatal inflammation was only associated with a lower ratio in the chorioamnionitis group compared with controls on day 3. A reason for this difference could be the lower birth weight in the chorioamnionitis group, which was shown to correlate inversely with endostatin levels by Janer *et al.* (15) and us. However, one of the limitations of our study is the small size of the chorioamnionitis group, which makes interpretation difficult.

On days 1, 3, and 15 the ratio angiopoietin-1/endostatin of infants who died or developed BPD was significantly lower than for infants without BPD. All infants died before day 28 of life making the diagnosis of BPD impossible. As they had the lowest gestational age and birth weights in our cohort (Table 2), both being independent risk factors for BPD, we combined the outcomes death and BPD for analysis. However,

looking only at survivors still yielded lower ratios with BPD on days 1 and 3. Infants with moderate BPD or death showed lower ratios on days 3 and 15 compared with the remainder. We cannot conclude any causal relationship from this association. However, there is evidence that angiogenesis is a prerequisite for normal alveolar development (4,44). A shift of the balance between angiopoietin-1 and endostatin under the influence of postnatal factors like mechanical ventilation, infection, or oxidative stress, might be part of the concert of angiogenic and angiostatic factors, which play roles in the development of BPD (6,29,39,45).

Our clinical prospective study has several limitations. The associations that we found do not prove a cause-and-effect relationship. Our patient number is small, which precludes control for potential confounders, as stated earlier. In addition, we did not assess activities of the two mediators but only concentrations. However, the wide variations of our values are consistent with those reported in comparable studies (15,38,39).

Our data from a cohort of ventilated extremely preterm infants suggested that a decrease of angiopoietin-1 and endostatin after systemic fetal inflammatory response could predispose these infants for further lung injury, aggravated by postnatal factors. Infants who developed BPD or died showed a temporary decrease of the ratio between the mediators, potentially playing a role for disturbed postnatal pulmonary development. The precise role of both angiopoietin-1 and endostatin for pulmonary morbidity in extremely preterm infants deserves further confirmation from clinical investigations with large sample sizes and appropriate animal models.

REFERENCES

1. Speer CP 2006 Role of inflammation in the evolution of bronchopulmonary dysplasia. *Drug Discov Today Dis Mech* 3:409–414
2. Husain AN, Siddiqui NH, Stocker JT 1998 Pathology of arrested acinar development in postsurfactant bronchopulmonary dysplasia. *Hum Pathol* 29:710–717
3. Kallapur SG, Bachurski CJ, Le Cras TD, Shubhada NJ, Ikegami M, Jobe AH 2004 Vascular changes after intra-amniotic endotoxin in preterm lamb lungs. *Am J Physiol Lung Cell Mol Physiol* 287:L1178–L1185
4. Jakkula M, Le Cras TD, Gebb S, Hirth KP, Tuder RM, Voelkel NF, Abman SH 2000 Inhibition of angiogenesis decreases alveolarization in the developing rat lung. *Am J Physiol Lung Cell Mol Physiol* 279:L600–L607
5. Thebaud B 2007 Angiogenesis in lung development, injury and repair: implications for chronic lung disease of prematurity. *Neonatology* 91:291–297
6. Bhatt AJ, Pryhuber GS, Huyck H, Watkins RH, Metlay LA, Maniscalco WM 2001 Disrupted pulmonary vasculature and decreased vascular endothelial growth factor,

- Flt-1, and Tie-2 in human infants dying with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 164:1971–1980
7. Lassus P, Turanlahti M, Heikkilä P, Andersson LC, Nupponen I, Sarnesto A, Andersson S 2001 Pulmonary vascular endothelial growth factor and Flt-1 in fetuses, in acute and chronic lung disease, and in persistent pulmonary hypertension of the newborn. *Am J Respir Crit Care Med* 164:1981–1987
8. Makinde T, Agrawal DK 2008 Intra and extra-vascular trans-membrane signaling of angiopoietin-1-Tie2 receptor in health and disease. *J Cell Mol Med* 12:810–828
9. Hughes DP, Marron MB, Brindle NP 2003 The antiinflammatory endothelial tyrosine kinase Tie2 interacts with a novel nuclear factor-kappaB inhibitor ABIN-2. *Circ Res* 92:630–636
10. Pizurki L, Zhou Z, Glynos K, Roussos C, Papapetropoulos A 2003 Angiopoietin-1 inhibits endothelial permeability, neutrophil adherence and IL-8 production. *Br J Pharmacol* 139:329–336
11. Abdollahi A, Hahnfeldt P, Maercker C, Grone HJ, Debus J, Ansgor W, Folkman J, Hlatky L, Huber PE 2004 Endostatin's antiangiogenic signaling network. *Mol Cell* 13:649–663
12. Shichiri M, Hirata Y 2001 Antiangiogenesis signals by endostatin. *FASEB J* 15:1044–1053
13. Schmidt A, Wenzel D, Ferring I, Kazemi S, Sasaki T, Hescheler J, Timpl R, Addicks K, Fleischmann BK, Bloch W 2004 Influence of endostatin on embryonic vasculo- and angiogenesis. *Dev Dyn* 230:468–480
14. Schmidt A, Addicks K, Bloch W 2004 Opposite effects of endostatin on different endothelial cells. *Cancer Biol Ther* 3:1162–1166; discussion 1167–1168
15. Janer J, Andersson S, Haglund C, Lassus P 2007 Pulmonary endostatin perinatally and in lung injury of the newborn infant. *Pediatrics* 119:e241–e246
16. D'Alquen D, Kramer BW, Seidenspinner S, Marx A, Berg D, Groneck P, Speer CP 2005 Activation of umbilical cord endothelial cells and fetal inflammatory response in preterm infants with chorioamnionitis and funisitis. *Pediatr Res* 57:263–269
17. Kramer BW, Kaemmerer U, Kapp M, Herbst D, Marx A, Berg D, Groneck PA, Speer CP 2005 Decreased expression of angiogenic factors in placentas with chorioamnionitis after preterm birth. *Pediatr Res* 58:607–612
18. Lau J, Magee F, Qiu Z, Hoube J, Von Dadelszen P, Lee SK 2005 Chorioamnionitis with a fetal inflammatory response is associated with higher neonatal mortality, morbidity, and resource use than chorioamnionitis displaying a maternal inflammatory response only. *Am J Obstet Gynecol* 193:708–713
19. Matsuda T, Nakajima T, Hattori S, Hanatani K, Fukazawa Y, Kobayashi K, Fujimoto S 1997 Necrotizing funisitis: clinical significance and association with chronic lung disease in premature infants. *Am J Obstet Gynecol* 177:1402–1407
20. Jobe AH, Bancalari E 2001 Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 163:1723–1729
21. Rogers BB, Alexander JM, Head J, McIntire D, Leveno KJ 2002 Umbilical vein interleukin-6 levels correlate with the severity of placental inflammation and gestational age. *Hum Pathol* 33:335–340
22. Watts CL, Bruce MC 1995 Comparison of secretory component for immunoglobulin A with albumin as reference proteins in tracheal aspirate from preterm infants. *J Pediatr* 127:113–122
23. Thomas W, Seidenspinner S, Kawczynska-Leda N, Kramer BW, Chmielnicka-Kopaczyn M, Marx A, Szymankiewicz M, Speer CP 2008 Systemic fetal inflammation and reduced concentrations of macrophage migration inhibitory factor in tracheobronchial aspirate fluid of extremely premature infants. *Am J Obstet Gynecol* 198:64.e1–e6
24. Pacora P, Chaiworapongsa T, Maymon E, Kim YM, Gomez R, Yoon BH, Ghezzi F, Berry SM, Qureshi F, Jacques SM, Kim JC, Kadar N, Romero R 2002 Funisitis and chorionic vasculitis: the histological counterpart of the fetal inflammatory response syndrome. *J Matern Fetal Neonatal Med* 11:18–25
25. Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, Jun JK 2000 The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. *Am J Obstet Gynecol* 183:1124–1129
26. Ristimäki A, Narko K, Enholm B, Joukov V, Alitalo K 1998 Proinflammatory cytokines regulate expression of the lymphatic endothelial mitogen vascular endothelial growth factor-C. *J Biol Chem* 273:8413–8418
27. Maniscalco WM, Watkins RH, Pryhuber GS, Bhatt A, Shea C, Huyck H 2002 Angiogenic factors and alveolar vasculature: development and alterations by injury in the very premature baboons. *Am J Physiol Lung Cell Mol Physiol* 282:L811–L823
28. De Paepe ME, Patel C, Tsai A, Gundavarapu S, Mao Q 2008 Endoglin (CD105) up-regulation in pulmonary microvasculature of ventilated preterm infants. *Am J Respir Crit Care Med* 178:180–187
29. Thebaud B, Ladha F, Michelakis ED, Sawicka M, Thurston G, Eaton F, Hashimoto K, Harry G, Haromy A, Korbutt G, Archer SL 2005 Vascular endothelial growth factor gene therapy increases survival, promotes lung angiogenesis, and prevents alveolar damage in hyperoxia-induced lung injury: evidence that angiogenesis participates in alveolarization. *Circulation* 112:2477–2486
30. Watterberg KL, Demers LM, Scott SM, Murphy S 1996 Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. *Pediatrics* 97:210–215
31. Kramer BW, Kramer S, Ikegami M, Jobe AH 2002 Injury, inflammation, and remodeling in fetal sheep lung after intra-amniotic endotoxin. *Am J Physiol Lung Cell Mol Physiol* 283:L452–L459
32. Ikegami M, Jobe AH 2002 Postnatal lung inflammation increased by ventilation of preterm lambs exposed antenatally to *Escherichia coli* endotoxin. *Pediatr Res* 52:356–362
33. Ekekezie II, Thibeault DW, Rezaiekhaliq MH, Norberg M, Mabray S, Zhang X, Truong WE 2003 Endostatin and vascular endothelial growth factor (VEGF) in piglet lungs: effects of inhaled nitric oxide and hyperoxia. *Pediatr Res* 53:440–446
34. Galambos C, deMello DE 2007 Molecular mechanisms of pulmonary vascular development. *Pediatr Dev Pathol* 10:1–17
35. Levy M, Maurey C, Chailley-Heu B, Martinovic J, Jaubert F, Israel-Biet D 2005 Developmental changes in endothelial vasoactive and angiogenic growth factors in the human perinatal lung. *Pediatr Res* 57:248–253
36. Ambalavanan N, Novak ZE 2003 Peptide growth factors in tracheal aspirates of mechanically ventilated preterm neonates. *Pediatr Res* 53:240–244
37. Andersson S, Merritt RA, Orpana A, Viinikka L, Ylikorkala O 1997 High endothelin-1 in the airways of preterm infants is associated with less severe respiratory distress during the early postnatal period. *Pediatrics* 99:545–547
38. D'Angio CT, Maniscalco WM, Ryan RM, Avissar NE, Basavegowda K, Sinkin RA 1999 Vascular endothelial growth factor in pulmonary lavage fluid from premature infants. Effects of age and dexamethasone. *Biol Neonate* 76:266–273
39. Lassus P, Ristimäki A, Ylikorkala O, Viinikka L, Andersson S 1999 Vascular endothelial growth factor in human preterm lung. *Am J Respir Crit Care Med* 159:1429–1433
40. Kinsella JP, Greenough A, Abman SH 2006 Bronchopulmonary dysplasia. *Lancet* 367:1421–1431
41. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, Bauer CR, Donovan EF, Korones SB, Laptook AR, Lemons JA, Oh W, Papile LA, Shankaran S, Stevenson DK, Tyson JE, Poole WK; NICHD Neonatal Research Network 2007 Trends in neonatal morbidity and mortality for very low birthweight infants. *Am J Obstet Gynecol* 196:147.e1–e8
42. Dzikowska-Bartkowiak B, Waszykowska E, Dzikowska-Zaboroszczyk E, de Graft-Johnson JE, Zalewska A, Luczynska M, Nowak D 2006 Decreased ratio of circulatory vascular endothelial growth factor to endostatin in patients with systemic sclerosis—association with pulmonary involvement. *Clin Exp Rheumatol* 24:508–513
43. Distler JH, Hirth A, Kurowska-Stolarska M, Gay RE, Gay S, Distler O 2003 Angiogenic and angiostatic factors in the molecular control of angiogenesis. *Q J Nucl Med* 47:149–161
44. Stenmark KR, Abman SH 2005 Lung vascular development: implications for the pathogenesis of bronchopulmonary dysplasia. *Annu Rev Physiol* 67:623–661
45. Thebaud B, Abman SH 2007 Bronchopulmonary dysplasia: where have all the vessels gone? roles of angiogenic growth factors in chronic lung disease. *Am J Respir Crit Care Med* 175:978–985