Cord Blood Biomarkers of Placental Maternal Vascular Underperfusion Predict Bronchopulmonary Dysplasia-Associated Pulmonary Hypertension

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Objective To assess whether cord blood biomarkers associated with placental maternal vascular underperfusion (MVU) are predictive of bronchopulmonary dysplasia-associated pulmonary hypertension (BPD-PH).

Study design Premature infants enrolled in a longitudinal cohort study were randomly sampled from 4 gestational age strata (n = 190, range 23-36 weeks). Fifteen factors from a human angiogenesis panel were measured in cord blood using multiplex immunoassay. Multivariate linear regression was used to compare biomarker levels according to placental histologic MVU, taking into account acute/chronic inflammation and fetal vascular pathology. Biomarkers associated with MVU were further evaluated in the subgroup of extremely low gestational age infants (gestational age ≤ 28 weeks; n = 48), and measured by enzyme-linked immunoassay in an additional 39 infants to determine associations with BPD (defined using the National Institutes of Health workshop criteria) and PH (identified by echocardiogram at 36 weeks of gestation).

Results Cord blood placental growth factor (PIGF), granulocyte-colony stimulating factor (G-CSF), and vascular endothelial growth factor-A were decreased with MVU ($P < .003$), and decreased with BPD-PH ($P < .05$). The findings were validated for PIGF and G-CSF in 39 additional extremely low gestational age infants. In the combined group (n = 87), PIGF was decreased in infants with BPD-PH (n = 21) versus controls without PH (median 3 pg/mL [IQR 2-7] vs median 15 pg/mL [IQR 6-30], respectively; $P < .001$). G-CSF was similarly decreased with BPD-PH (median, 55 pg/mL [IQR 38-85] vs median 243 pg/mL [IQR 48-1593], respectively; $P = .001$). Receiver operator curve analysis revealed that decreased PIGF and G-CSF were predictive of BPD-PH (area under the curve 0.83 and 0.76, respectively).

Conclusions Cord blood angiogenic factors that are decreased with placental MVU may serve as predictors of BPD-PH. *(J Pediatr 2017;185:33-41).*

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Maternal vascular underperfusion (MVU) is a group of placental histologic lesions seen in births complicated by preeclampsia and intrauterine growth restriction. Findings of MVU include placental vascular bed abnormalities such as fibrinoid necrosis with acute atherosis (FN/AA), and maldevelopment of the villous tree as in distal villous hypoplasia with small terminal villi (DVH/STV).[1] These aberrations in placental morphology are thought to be the result of abnormal placentation, with chronic malperfusion of the chorionic villi by the maternal vascular supply. Chronic fetal hypoxia owing to placental insufficiency, as indicated by the severity and extent of MVU may explain the reported associations between maternal preeclampsia and adverse infant outcomes.[2-4]

BW	Birth weight
BPD	Bronchopulmonary dysplasia
BPD-PH	Bronchopulmonary dysplasia-associated pulmonary hypertension
DVH/STV	Distal villous hypoplasia with small terminal villi
ELGAN	Extremely low gestational age
FN/AA	Fibrinoid necrosis with acute atherosis
FGR	Fetal growth restriction
GA	Gestational age
G-CSF	Granulocyte colony-stimulating factor
MVU	Maternal vascular underperfusion
PH	Pulmonary hypertension
PIGF	Placental growth factor
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor-A

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In a large epidemiologic study, we described the association between placental histologic MVU and the development of pulmonary hypertension (PH) in premature infants with bronchopulmonary dysplasia (BPD).[5] BPD, defined as persistent oxygen dependence at 36 weeks postmenstrual age,[6] affects up to one-third of extremely low gestational age (ELGAN) infants born at <28 weeks of gestation.[7-9] In 25%-40% of infants with BPD, pulmonary vascular disease presenting as PH also persists.[8] This entity, known as BPD-associated PH (BPD-PH) is characterized by more severe cardiopulmonary instability and worse long-term outcomes among survivors.[9,10] BPD-PH is associated with a 4-fold increased risk of death as compared with infants who have BPD without PH,[11,12] yet there are no reliable early biomarkers to distinguish risk.

There is increasing evidence that the pulmonary vascular maldevelopment characteristic of BPD-PH begins even before birth, and that it is mediated by chronic fetal hypoxia.[13] Placental MVU helps to identify infants exposed to such hypoxia. Correlation with systemic markers that can further enhance our understanding of how placental dysfunction impacts fetal lung angiogenesis are needed. Cord blood angiogenic factors that vary with placental histologic MVU are promising indicators, because cord blood is taken directly from the infant's circulation at the time of birth and is a direct correlate of placental function leading up to preterm birth.

The purpose of this study was 2-fold. First, we sought to identify cord blood biomarkers associated with placental MVU, taking into account gestational age (GA) and other important covariates. Our second objective was to determine whether MVU-associated biomarkers are in turn associated with later BPD-PH among the ELGAN infants. Building on our previous report that MVU is associated with the development of PH in infants with BPD,[5] we hypothesized that cord blood biomarkers associated with MVU are predictive of BPD-PH.

Methods

The patient sample was drawn from an ongoing longitudinal cohort study conducted at Prentice Women's Hospital in Chicago. Eligible patients of the parent study are live births ranging from 23 to 36 weeks of gestation. The study was approved by the Institutional Review Board at Northwestern University. Maternal informed consent was obtained before participation of all mothers and their babies. Cord blood is collected at birth, and placentas are sent for gross and histopathologic examination by a perinatal pathologist. Because the multiplex assay platform for the current study accommodated 190 samples in duplicate, we narrowed the sample size to include only singleton patients enrolled between January 2012 and December 2013. Multiple gestation births and infants with congenital anomalies, known genetic syndromes, congenital infections, and metabolic disorders were excluded. Only patients who survived to hospital discharge were included. To control for the influence of GA, we performed stratified sampling of this population (n = 564 infants), partitioning first into 4 strata based on completed weeks gestation at birth: (1) extremely preterm (23-28 weeks, n = 63), (2) very preterm (29-31 weeks, n = 108), (3) moderately preterm (32-34 weeks, n = 272), and (4) mildly preterm (35-36 weeks, n = 121). Simple random sampling was then performed within each strata to obtain 47-48 infants in each of the 4 GA groups, masked to all clinical and placental information. From this sample of 190 births, we identified biomarkers of placental MVU via multiplex immunoassay. After this first analysis, 13 additional ELGAN infants with BPD-PH were identified from the parent study and matched by GA with 13 infants with BPD only and 13 controls without BPD or PH. Cord blood plasma levels from this second cohort of 39 infants were measured via individual enzyme-linked immunoassay, then combined with biomarker data from ELGAN infants from the first cohort.

Maternal and infant data were collected prospectively per the parent study protocol onto standardized abstraction forms that included information on intrapartum management, pregnancy complications, and infant hospital course. GA was assessed with an algorithm based on last menstrual period and ultrasound imaging.[14] Preeclampsia and related complications were defined according to American College of Obstetricians and Gynecologists' criteria.[15] Fetal growth restriction (FGR) was defined as a birth weight (BW) of <10th percentile for GA based on Fenton growth curves.[16] BPD was defined by the National Institutes of Health consensus definition of oxygen requirement at 36 weeks postmenstrual age.[6] All infants who required oxygen at this time point received an echocardiogram evaluation, and PH status was determined according to an algorithm previously published for this cohort.[5,17]

Cord blood was obtained by labor and delivery staff into EDTA tubes, centrifuged for 10 minutes in a tabletop refrigerated centrifuge at 3000 rpm. Plasma was removed from the cell pellet and stored at −80°C until assay. Simultaneous measurement of 15 biomarkers was performed by sandwich immunoassays using Luminex xMAP platform in magnetic bead format. The multiplexed assay beads were obtained from a commercially available kit (Human Angiogenesis/Growth Factor Magnetic Bead Panel 1, HAGP1MAG-12K, EMD Millipore, Massachusetts). The 15 angiogenic proteins were selected from 17 analytes available for this platform based on comprehensive review of the literature for factors directly or indirectly associated with preeclampsia or FGR, that could biologically exert their effects through placental vascular disease: epidermal growth factor, hepatocyte growth factor, and heparin-binding epidermal growth factor[18]; angiopoietin-2, endoglin, vascular endothelial growth factor (VEGF), and placental growth factor (PIGF)[19]; granulocyte colony-stimulating factor (G-CSF) and interleukin-8[20]; endothelin-1[21]; leptin[22]; and fibroblast growth factor-1 and fibroblast growth factor-2.[23] Plasma samples were thawed on ice and prepared in 1:3 dilution. The samples were analyzed on the Luminex platform according to manufacturer's instructions. All samples were run in duplicate with standard curves for each marker and controls on each plate. Intra-assay and interassay coefficients of variation were <15% and <5%, respectively, based on absolute differences in concentrations for each analyte. For further evaluation of the MVU- associated biomarkers measured according to BPD-PH outcomes, individual assays were performed in 1:1 dilution