Plasma Asymmetric Dimethylarginine Levels Are Increased in Neonates with Bronchopulmonary Dysplasia-Associated Pulmonary Hypertension

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Objective To test the hypothesis that levels of the endogenous inhibitor of nitric oxide production, asymmetric dimethylarginine (ADMA), would be greater in preterm infants with bronchopulmonary dysplasia (BPD)-associated pulmonary hypertension (PH) than in infants with BPD alone.

Study design A case-control study of 23 patients with both BPD and PH (cases) and 95 patients with BPD but no evidence of PH (controls). Levels of ADMA were compared between cases and controls by *t* test.

Results Patients with both BPD and PH had greater plasma levels of ADMA than patients with BPD alone ($P = .04$). In samples drawn before 28 days of life, greater levels of ADMA were again found in cases compared with controls ($P = .02$). The plasma arginine-to-ADMA ratio was lower in cases than in controls ($P = .03$), suggesting a greater likelihood of inhibition of nitric oxide production in patients with both BPD and PH than in patients with BPD alone.

Conclusion In this neonatal BPD cohort, ADMA levels are increased in patients with BPD who develop PH. We speculate that ADMA may be both a biomarker and a potential therapeutic target for preterm infants with BPD-associated PH. *(J Pediatr 2015;166:230-3)*.

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Bronchopulmonary dysplasia (BPD) is the most common pediatric chronic lung disease.[1] Pulmonary hypertension (PH) is a complication of BPD, with a prevalence estimated between 25% and 37%.[2-4] PH is associated with an increase in morbidity and mortality.[5,6] Currently, not only is it difficult to diagnose PH in BPD, but there are no clinical tests for predicting which patients with BPD will develop PH. PH in BPD is likely the result of abnormal vasculature development in the preterm lung.[7] Both the decreased surface area and vasoconstriction of the pulmonary vasculature can contribute to the increased vascular resistance and greater pulmonary arterial pressures in patients with both BPD and PH.

Nitric oxide (NO) is produced from L-arginine by NO synthase (NOS), and NO is central in maintaining the normal low pulmonary vasculature resistance seen. In patients with certain forms of PH, endogenous NO production is decreased.[8-11] Therefore, the regulation of NO is potentially both a biomarker and a therapeutic target in BPD-associated PH. The production of NO can be inhibited by asymmetric dimethylarginine (ADMA). Currently, little is known regarding the role of ADMA in neonatal disease.

ADMA is formed by the methylation of arginine residues contained in proteins by the protein arginine methyltransferases (PRMT), and subsequent proteolysis results in the release of methylated arginines, including ADMA. ADMA is degraded primarily by dimethylarginine dimethylaminohydrolase (DDAH). ADMA competes with L-arginine for the active site of NOS, and when ADMA is bound to NOS, NO production by NOS is inhibited. Normally, the balance between production of ADMA and its degradation by DDAH results in low levels of ADMA and relatively little inhibition of NOS.[12] In cardiovascular and renal disease, however, levels of ADMA are increased.[9,13] Therefore, we tested the hypothesis that plasma levels of ADMA would be greater in preterm patients with both BPD and PH than in patients with BPD without evidence of PH.

ADMA	Asymmetric dimethylarginine
BPD	Bronchopulmonary dysplasia
DDAH	Dimethylarginine dimethylaminohydrolase
NICU	Neonatal intensive care unit
NO	Nitric oxide
NOS	Nitric oxide synthase
PH	Pulmonary hypertension
PRMT	Protein arginine methyltransferases

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Methods

The Institutional Review Board of Nationwide Children's Hospital approved this study; informed consent was obtained from patients and their families. All patients admitted to Nationwide Children's Hospital neonatal intensive care units (NICUs) after September 1, 2009 with the diagnosis of BPD were eligible for this study. BPD was defined according to the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development workshop statement as a supplemental oxygen requirement at 28 days of life.[14] Enrollment, clinical data abstraction, and specimen collection was completed through the Ohio Perinatal Research Network.

Patients with both BPD and PH were identified as those with evidence of abnormally increased pulmonary arterial pressure on echocardiography with a structurally normal heart. Increased pulmonary arterial pressure on echocardiography was defined by the presence of any of the following 4 criteria: (1) right ventricular hypertrophy; (2) flattening of the intraventricular septum; (3) tricuspid regurgitation in the absence of pulmonary stenosis; and/or (4) increased right ventricular pressure as estimated by tricuspid regurgitation jet velocity.[2,15-17] Infants with BPD who did not have PH according to these criteria were considered controls. At the time of this study, there was no screening protocol for PH in patients with BPD, and therefore 62% of the control population was screened with echocardiography for PH. Indications for inhaled NO were variable, including support of lung development, as well as severe BPD. Patients with congenital heart disease (except for patent ductus arteriosus and/or atrial septal defect) were excluded. Patients with anatomical causes of PH, including diaphragmatic hernia or other causes of lung hypoplasia, also were excluded.

Blood samples were collected from all patients at enrollment. Whole blood samples were immediately centrifuged and the plasma collected and stored at −80°C. Concentrations of metabolites (citrulline, arginine, ornithine, and proline) and ADMA, were determined using reverse-phase high-performance liquid chromatography and expressed in concentration (μM) as previously described.[9] In brief, an analysis was performed on a Shimadzu high-performance liquid chromatography (Shimadzu Corp, Kyoto, Japan) equipped with a RF-10AXL fluorescence detector and Class VP 7.3 data analysis software. Fluorescence was monitored at an excitation wavelength of 250 nm and an emission wavelength of 395 nm.[9]

Statistical Analyses

Data are reported as mean ± SD, or as number and percent. Demographics and clinical characteristics of cases (BPD and PH) and controls (BPD alone) were compared using χ^2 test for categorical data and Student *t* test for continuous data. Blood metabolite levels were compared between study populations by Student *t* test. $P < .05$ was considered statistically significant.

Results

Among 122 patients with BPD enrolled in the study, 23 had both BPD and PH (cases), 95 had BPD alone (controls), and 4 patients met criteria for exclusion. The diagnosis of PH in patients with BPD was made by one of the following predominant findings on echocardiography: right ventricular hypertrophy (39%), flattening of the intraventricular septum (9%), tricuspid regurgitation in the absence of pulmonary stenosis (35%), or increased right ventricular pressure (17%). There were no differences in clinical characteristics between cases and controls (**Table I**). These infants were born very preterm and had very low birth weight, and as perhaps expected for an all referral NICU, a number of these patients were admitted relatively late in their clinical course. Of the patients with both BPD and PH, 65% were discharged on supplemental oxygen therapy, 22% of infants were discharged on furosemide, and 13% of infants received NO therapy during their hospital stay. We found no differences between cases and controls in respiratory treatments after admission to the NICU (**Table II**).

Plasma samples from 118 patients were analyzed for ADMA, arginine, citrulline, ornithine, and proline levels (**Table III**). The average sample day of life for patients with both BPD and PH was 54 ± 40 days and 38 ± 22 days for those with BPD alone. The plasma levels of ADMA were found to be approximately 2-fold greater in patients with both BPD and PH than in patients with BPD alone. Plasma levels of citrulline, arginine, ornithine, and proline were not different between patients with both BPD and PH and patients with BPD alone (**Table III**). We found no difference in citrulline-to-ornithine ratio between cases and controls; however, the plasma arginine-to-ADMA ratio was nearly 3 times lower in patients with both BPD and PH than patients with BPD alone. For plasma ADMA levels,

Table I. Demographic and clinical characteristics

	BPD alone (n = 95)	BPD + PH (n = 23)	*P* value
Gestational age, wk	28.2 ± 3.7	27.5 ± 3.9	.42
Birth weight, g	1179 ± 597	959 ± 517	.09
Female, n (%)	31 (33)	8 (35)	.84
White, n (%)	68 (72)	18 (78)	.41
Birth head circumference, cm	26 ± 4	24 ± 3	.07
5-min Apgar	6 ± 2	6 ± 2	.35
Admission age, d	28 ± 48	46 ± 88	.36
PDA, n (%)	41 (43)	9 (39)	.85
IVH, n (%)	20 (21)	8 (35)	.17
Hydrocephalus, n (%)	2 (2)	1 (4)	.54
NEC, n (%)	4 (4)	1 (4)	.98
ROP, n (%)	11 (12)	2 (9)	.69
Pneumothorax, n (%)	2 (2)	2 (9)	.13
NO, n (%)	7 (7)	3 (13)	.40
Postadmission, steroids, n (%)	31 (33)	11 (48)	.17
Caffeine, n (%)	57 (60)	15 (65)	.65
Surfactant, n (%)	61 (64)	15 (65)	.73
Discharge weight, g	3433 ± 1278	3677 ± 1351	.44

IVH, intraventricular hemorrhage; *NEC*, necrotizing enterocolitis; *PDA*, patent ductus arteriosus; *ROP*, retinopathy of prematurity.