Published in final edited form as:
Pediatr Cardiol. 2013 January ; 34(1): 149–154. doi:10.1007/s00246-012-0404-7.

Association Between Blood Spot Transforming Growth Factor-β and Patent Ductus Arteriosus in Extremely Low-Birth Weight Infants

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Abstract

Permanent ductal closure involves anatomic remodeling, in which transforming growth factor (TGF)-β appears to play a role. Our objective was to evaluate the relationship, if any, between blood spot TGF-β on day 3 and day 7 of life and patent ductus arteriosus (PDA) in extremely low birth weight (ELBW) infants. Prospective observational study involving ELBW infants (n = 968) in the National Institute of Child Health and Human Development Neonatal Research Network who had TGF-β measured on filter paper spot blood samples using a Luminex assay. Infants with a PDA (n = 493) were significantly more immature, had lower birth weights, and had higher rates of respiratory distress syndrome than those without PDA (n = 475). TGF-β on days 3 and 7 of life, respectively, were significantly lower among neonates with PDA (median 1,177 pg/ml [range 642–1,896]; median 1,386 pg/ml [range 868–1,913]) compared with others without PDA (median 1,334 pg/ml [range 760–2,064]; median 1,712 pg/ml [range 1,014–2,518 pg/ml]). The significant difference persisted when death or PDA was considered a composite outcome. TGF-β levels were not significantly different among subgroups of infants with PDA who were not treated (n = 51) versus those who were treated medically (n = 283) or by surgical ligation (n = 159). TGF-β was not a significant predictor of death or PDA (day 3 odds ratio [OR] 0.99, 95 % confidence interval [CI] 0.83–1.17; day 7 OR 0.88, 95 % CI 0.74–1.04) on adjusted analyses. Our results suggest that blood spot TGF-β alone is unlikely to be a reliable biomarker of a clinically significant PDA or its responsiveness to treatment.

Keywords

Transforming growth factor; Patent ductus arteriosus; Preterm; Neonate

Introduction

Patent ductus arteriosus (PDA) is a frequent occurrence in preterm infants, with reported rates of 40–55 % among those born at<29 weeks' gestation [8, 12]. The process of ductal closure occurs in two phases: (1) functional constriction soon after birth followed by anatomic permanent closure; and (2) neointimal cushion formation by migration of smooth muscle cells from the muscle media into the subendothelial space, which eventually occlude the ductal lumen [5]. Anatomic remodeling appears to be initiated by ductal wall hypoxia, although the precise mechanisms remain unclear. Studies on animal models suggest that transforming growth factor (TGF) contributes to the mechanisms of anatomic ductal closure [2, 10, 11, 15]. Our objective in the current exploratory analyses was to examine the relationship, if any, between blood spot TGF-β on days 3 and 7 of life and PDA in extremely low-birth weight (ELBW) infants.

Patients and Methods

Study Population

This is a secondary analyses of data collected from preterm infants who participated in the Inflammatory Cytokines and Neurodevelopmental Outcomes in Extremely Low Birth Weight Infants study of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) multicenter Neonatal Research Network (NRN) [3]. The study was approved by the Institutional Review Boards of all participating centers, and informed parental consent was obtained. Preterm infants with birth weights between 401 and

1,000 g, in whom TGF-β levels were obtained at least once on either day 3 and 7, were included.

Study Procedures

Clinically significant PDA was defined as clinical evidence of left-to-right PDA shunt, i.e., a continuous murmur, hyperdynamic precordium, bounding pulses, wide pulse pressure, congestive heart failure, increased pulmonary vascularity or cardiomegaly by chest radiograph, and/or increased oxygen requirement or echocardiographic evidence of left-to-right ductal shunting. Although not required by the NRN, all of the NRN centers confirmed clinical symptoms with echocardiography for the presence of PDA. Infants without PDA comprised the comparison control group. Whole-blood spots, dried on filter paper, were obtained on days 3 ± 1 and 7 ± 1 of life from indwelling arterial/venous lines or heel sticks, allowed to dry at room temperature, and stored at −20 °C. TGF-β measurement was by xMAP (multiplex Luminex) assay (Luminex, Austin, TX) with reported intra and inter-assay coefficients of variation of 6.8 and 18 %, respectively, and constant TGF-β amounts during storage [9].

Data Analysis

Statistical analyses (SAS version 9.1.3 [SAS, Cary, NC]) included Wilcoxon test for unadjusted comparisons of variables between two groups of infants with and without clinically significant PDA. For comparisons of TGF-β blood spot concentrations between subgroups of infants with PDA who were not treated and those who were treated medically or by surgical ligation, Kruskal–Wallis nonparametric tests were used. Logistic regression was used to determine the association between TGF-β and a PDA after adjusting for covariates. Statistical significance was set at a $p < 0.05$.

Results

Demographic Characteristics

Among 1,067 extremely preterm infants who participated in the primary cytokines study of the NICHD multicenter NRN, 968 with a TGF-β sample at either time point (day 3 or day 7 of life) were included. Figure 1 depicts the study cohort and outcomes, and Table 1 shows that infants with PDA were significantly more premature, had lower mean birth weight, and had a higher incidence of respiratory distress syndrome (RDS) requiring surfactant (all $p < 0.0001$).

TGF-β and PDA

Blood spot TGF-β concentrations on days 3 and 7 were significantly lower in the PDA group ($n = 493$) compared with the no-PDA group ($n = 475$), although this was with considerable overlap ($p < 0.01$) (Table 2). When death and/or PDA, respectively, was used as a composite outcome, TGF-β levels at both time points were significantly lower in those who died or had a PDA than among survivors without PDA (day 3 = 1,182 (range 643–1,868) pg/ml vs. 1,370 (range 761–2,079) pg/ml [$p < 0.005$] and day 7 = 1,382 (range 865–1,923) pg/ml vs. 1,749 (range 1,046–2,550) pg/ml [$p < 0.0001$]). Among subgroups of surviving infants, the difference remained significant ($p < 0.0001$) on day 7 but not on day 3. There were no significant differences in blood spot TGF-β levels at either time point between subgroups of infants with PDA who were not treated and those who were treated medically or by surgical ligation (Table 3). Multivariable logistic regression was used to examine the relationship between TGF-β and a PDA as well as a composite outcome of death and/or PDA using gestation, birth weight, sex, race, antenatal (AN) steroids, surfactant doses, center, and early-onset sepsis as covariates. Gestation, AN steroids, surfactant doses,

and center were statistically significant covariates, whereas TGF-β levels on day 3 (odds ratio [OR] 0.99; 95 % confidence interval [CI] 0.83–1.17) or day 7 (OR 0.88; 95 % CI 0.74–1.04) was not (Table 4).

Discussion

TGF-β is a potent modulator of vascular smooth-muscle cell migration and is thought to play a role in ductal closure [2, 6]. TGF-β 1 is found in the wall of the fetal ductus and increases during postnatal closure [2, 11]. In full-term lambs, TGF-β protein and mRNA are present in low levels in late gestation with enhanced expression in the neointima and outer muscle media within 24 h of birth and a progressive increase during the next 10 days [11]. TGF-β has been shown to regulate increased ductus arteriosus endothelial glycoaminoglycan synthesis, which is associated with intimal proliferation [2]. In a fetal lamb model, intimal cushion formation was shown to be secondary to increased hyaluron synthesis, a TGF-β-dependent process [15]. Certain genetic conditions, such as Loeys Dietz syndrome, associated with mutations of the TGFBR2 gene may manifest in the neonatal period with clinical features, including PDA with or without ductal aneurysm [14].

There has been only a single previous study exploring the relationship between TGF-β and PDA in preterm (<32 weeks) infants. Median bronchoalveolar lavage fluid concentrations of both vascular endothelial growth factor (VEGF) and TGF-β1 on day 3, respectively, were found to be significantly greater in the PDA group ($n = 17$) (3,319 vs. 1,514 [$p = 0.02$]; 18,692 vs. 13,057 [$p = 0.03$]) compared with controls ($n = 23$) [13]. The investigators postulated that greater VEGF may be central to the acute injury phase, determining persistence of the PDA, whereas increased TGF-β1 may represent an attempted response to close the ductus.

Our exploratory analyses on a large cohort of preterm infants is the first attempt to uncover a possible correlation between blood TGF-β levels and PDA and calls for further focused investigation. The lack of demonstration of an independent association with clinically significant PDA is probably because blood TGF, although most easily accessible, may not reflect ductal-tissue concentrations or local function. The significantly lower blood TGF-β concentrations noted in the PDA group can probably be ascribed to the lower gestation and birth weight in this group. Previous data have showed a moderate positive correlation between TGF-β in bronchoalveolar lavage fluid and gestation and birth weight [4].

Limitations

The limitations of our study are that TGF-β may be altered by factors such as maternal chorioamnionitis, bronchopulmonary dysplasia, and platelet count [1, 4, 7]. Our assay measured total TGF-β, not the subunits. We opted to evaluate blood spot TGF on days 3 and 7 of life, the period of anticipated anatomic ductal closure but we did not have data on the exact timing of diagnosis of PDA or its treatment. The definition of clinically significant PDA in our data set included echocardiographic findings of any left-to-right shunt across the PDA. We attempted to discriminate hemodynamically significant and nonsignificant PDA by comparing subgroups of infants with PDA who were not treated with those who were treated medically or by surgical ligation. We recognize, however, that we did not have stringent echocardiographic findings of left atrial enlargement or ductal size to support the diagnosis of hemodynamically significant PDA and that there are enormous practice variations in the decision to evaluate for and treat PDA.

Our results suggest that blood spot TGF-β alone is unlikely to be a reliable biomarker of clinically significant PDA. There remains an urgent need to identify objective biomarkers

for the occurrence, severity, and responsiveness of PDA, which in turn could lead to a more rational approach to its management.

Acknowledgments

Supported by grants from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the Department of Health and Human Services (Grants No. U10 HD21385, U10 HD40689, U10 HD 27871, U10 HD21373, U10 HD36790, U10 HD40461, U10 HD34216, U10 HD21397, U10 HD27904, U10 HD40492, U10 HD27856, U10 HD40521, U10 HD27853, U10 HD27880, U10 HD27851, and R03 HD054420) and from the National Institutes of Health (Grants No. GCRC M01 RR 08084, M01 RR 00125, M01 RR 00750, M01 RR 00070, M01 RR 0039-43, M01 RR 00039, and 5 M01 RR00044). The National Institutes of Health, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and the Centers for Disease Control and Prevention provided grant support for recruitment for 1999 through 2001 and data analysis for the Neonatal Research Network's Cytokines Study. The funding agencies provided overall oversight for study conduct, but all data analyses and interpretation were independent of the funding agencies. Data collected at participating NRN sites were transmitted to RTI International, the data-coordinating center (DCC) for the NRN, which stored, managed, and analyzed the data for this study. On behalf of the network, Abhik Das (DCC PI) and Scott A. McDonald (DCC statistician) had full access to all of the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. We are indebted to our medical and nursing colleagues as well as the infants and their parents who agreed to take part in this study.

Appendix: NICHD Neonatal Research Network

The following investigators participated in this study:

NRN Steering Committee Chair: Alan H. Jobe, MD PhD, University of Cincinnati.

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Emory University Children's Healthcare of Atlanta, Grady Memorial Hospital, and Emory Crawford Long Hospital (GCRC M01 RR39, U10 HD27851)—Ellen C. Hale, RN BS CCRC.

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RTI International (U01 HD36790)—W. Kenneth Poole, PhD; Betty K. Hastings; Kristin M. Zaterka-Baxter, RN BSN; Jeanette O'Donnell Auman, BS; Scott E. Schaefer, MS.

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Yale University Yale-New Haven Children's Hospital (GCRC M01 RR6022, U10 HD27871)—Patricia Gettner, RN; Monica Konstantino, RN BSN; JoAnn Poulsen, RN.

Abbreviations

PDA	Patent ductus arteriosus
ELBW	Extremely low birth weight

TGF Transforming growth factor

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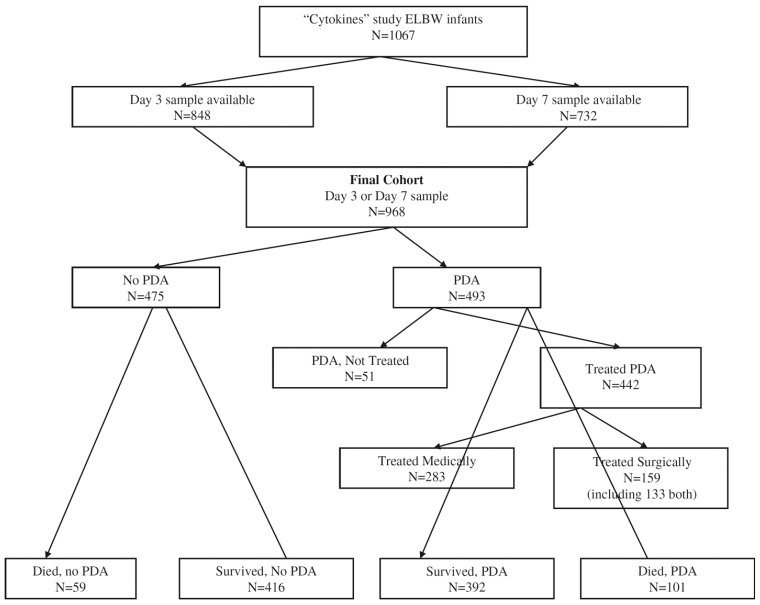

Fig. 1.
Flowchart of study cohort and outcomes

Table 1

Comparison of clinical characteristics of infants with and without PDA

Variable	PDA	No PDA	p [a]
	***N* (%) or mean (SD)**	***N* (%) or mean (SD)**	
	***N* = 493**	***N* = 415**	
Birth weight	734 ± 134	790 ± 138	<0.0001
GA (week)	25.3 ± 1.7	26.4 ± 2.0	<0.0001
Male sex	241 (49)	229 (48)	0.85
Black race	238 (48)	229 (48)	1.00
White race	248 (50)	239 (50)	
Other race	7 (1)	7 (1)	
AN steroids	365 (74)	376 (79)	0.07
5-min Apgar < 5	72 (15)	50 (11)	0.07
RDS[b]	480 (97)	428 (90)	<0.0001
Any surfactant	434 (88)	352 (74)	<0.0001
Surfactant (no. of doses)	1.7 ± 1.1	1.4 ± 1.1	<0.0001
Early-onset sepsis	10 (2)	6 (1)	0.45
Small for gestation	40 (8)	97 (20)	<0.0001

[a] p Values are from nonparametric Wilcoxon two-sample test (t approximation) for continuous variables and from Fisher's exact test for categorical variables

[b] RDS is defined as clinical features of RDS within age 24 h. We also have data for oxygen requirement from 6 to 24 h, need for respiratory support to 24 h, and abnormal chest X-ray within 24 h. Incorporating these items brings RDS to 100 % in both groups

Table 2

Median (IQR) TGF-β levels at different time points for infants with and without PDA

Plasma TGF-β (pg/ml)	**PDA**	**No PDA**
Median (IQR)	***N* = 493**	***N* = 475**
Day 3 *[a]	1177 (642–1896)	1334 (760–2064)
Day 7 **	1386 (868–1913)	1712 (1014–2518)

IQR interquartile range

[a] All *p* Values are from nonparametric Wilcoxon two-sample test (*t* approximation)

* $p < 0.01$

** $p < 0.0001$

Table 3

Median (IQR) TGF-β levels at different time points by type of treatment for PDA

Plasma TGF-β (pg/ml) Median (IQR)	PDA not treated *N* = 51	PDA treated medically *N* = 283	PDA treated surgically *N* = 159
Day 3 *	951 (641–1545)	1166 (636–1924)	1290 (659–1937)
Day 7 **	1228 (889–1484)	1476 (953–1996)	1242 (811–1910)

IQR interquartile range

* $p = 0.27$ (Kruskal–Wallis test); $p = 0.20$ (median test)

** $p = 0.09$ (Kruskal–Wallis test); $p = 0.04$ (median test)

Table 4

OR and 95 % CI (after adjusting for covariates) of day 3 and 7 TGF-β levels in the prediction of death or PDA

	p	OR	95 % CI
Day 3			
TGF-β (1000 pg/ml)	0.89	0.99	0.83–1.17
GA (week)	<0.0001	0.66	0.58–0.74
Male sex	0.96	0.99	0.71–1.38
Race	0.69		
White versus black	0.47	1.15	0.80–1.65
Other versus black	0.57	1.66	0.29–9.43
Birth weight	0.15	0.90	0.77–1.04
AN steroids	0.003	0.53	0.34–0.81
No. of surfactant doses (severity of RDS)	<0.0001	1.52	1.28–1.81
Early-onset sepsis	0.31	2.32	0.46–11.65
Center	<0.0001	Varies	
Day 7			
TGF-β (1,000 pg/ml)	0.13	0.88	0.74–1.04
GA (week)	<0.0001	0.66	0.58–0.74
Male sex	0.15	0.77	0.53–1.10
Race	0.47		
White versus black	0.63	1.10	0.74–1.64
Other versus black	0.31	0.41	0.07–2.35
Birth weight	0.14	0.89	0.75–1.04
AN steroids	0.01	0.57	0.36–0.89
No. of surfactant doses (severity of RDS)	<0.0001	1.49	1.23–1.80
Early-onset sepsis	0.43	1.98	0.37–10.55
Center	<0.0001	Varies	