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ARTICLE

NTproBNP as a surrogate biomarker for early screening of pulmonary hypertension in preterm infants with bronchopulmonary dysplasia

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Abstract

Objective Pulmonary hypertension (PH) is a known complication of bronchopulmonary dysplasia (BPD). This study aimed to determine the utility of serial N-Terminal pro-Brain Natriuretic Peptide (NTproBNP) levels in the screening of BPD associated PH (BPD-PH) in preterm infants.

Study design Infants with birth weight <1500 g and <30 week corrected gestational age (CGA) were followed with serial NTproBNP levels and echocardiograms (ECHO). They were divided into control, BPD and BPD-PH groups. Statistical analyses included repeated measures analysis of variance and receiver operator curve (ROC) generation.

Results Infants in the BPD-PH and BPD group had significantly elevated NTproBNP levels as compared to the control group. ROC curves for NTproBNP at 28 weeks CGA provided a cut-point of 2329 pg/ml and 578.1 pg/ml for detection of BPD-PH and BPD, respectively.

Conclusions NTproBNP appears to be a good screening tool to determine the onset of BPD-PH as early as 28 weeks CGA.

Introduction

Pulmonary hypertension (PH) is defined as a mean pulmonary artery pressure >25 mmHg after the first 3 months of life [1]. It is a well-known complication of neonatal respiratory diseases including bronchopulmonary dysplasia (BPD). Almost 18–25% of preterm infants with moderate to severe BPD develop PH (BPD-PH) [2, 3] with high morbidity and mortality (up to 38%) [4, 5]. The definitive diagnosis of PH is usually done by measuring the pulmonary artery pressure during cardiac catheterization, which may not be feasible in the majority of cases [5]. Most centers rely on the echocardiographic (ECHO) parameters for the diagnosis of PH although no concrete guidelines exist for the diagnosis of PH in the neonatal period. ECHO has its own limitations that include its cost, the need for experienced personnel to perform and interpret and sometimes its subjectivity [6].

For these reasons, various biochemical markers have been proposed as potential screening tools in the diagnosis and management of BPD-PH in preterm infants. N terminal pro B-type natriuretic peptide (NTproBNP) is synthesized and secreted mainly by the ventricular myocardium under conditions of sustained volume and pressure overload [7]. Montgomery et al. [8] showed good correlation of NTproBNP as a surrogate biomarker for the diagnosis of BPD-PH in preterm infants but Koenig et al. failed to demonstrate a similar correlation [9]. The recent American Heart Association Guidelines recommend that brain natriuretic peptide (BNP) or NTproBNP be measured at diagnosis and during follow-up to supplement clinical decision (Class I; Level of Evidence B) [1].

In a recent meta-analysis Al-Ghanem et al. [10] found higher odds of mortality and morbidity in infants with BPD-PH and they further suggested the need for routine screening for PH in preterm infants with BPD. However, without evidence of any benefit of early screening and

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treatment, such screening programs may be best suited in the context of properly designed prospective studies by using some screening biomarkers of BPD-PH. The purpose of our study was to determine the utility of serial NTproBNP levels in the screening of early BPD-PH in preterm infants.

Methods

Study design

This was a prospective observational study. After the institutional review board approval and parental informed consent, we recruited very low birth weight infants (birth weight <1500 g and <30 weeks CGA) between February 2015 and February 2017. Infants with known chromosomal anomalies or congenital defects affecting the cardiorespiratory system were excluded.

Definitions

- *Gestational age:* calculated either from the 1st trimester ultrasound or last menstrual period which ever was earlier.
- *BPD:* defined as supplemental oxygen requirement and/or respiratory support at 36 weeks CGA.
- *PH:* defined as ECHO findings of pulmonary artery pressure >25 mmHg estimated by tricuspid regurgitant jet velocity, the presence of right ventricular hypertrophy and/or flattening of the inter-ventricular septum [11]. We classified an infant as having PH if all 3 ECHO's at 28, 32, and 36 weeks CGA showed evidence of PH.

Study groups

Infants meeting the inclusion criteria were divided into three groups:

(i) *BPD-PH group:* infants with BPD and ECHO evidence of PH.
(ii) *BPD group:* infants with BPD without ECHO evidence of PH.
(iii) *Control group:* infants without BPD and no evidence of PH by ECHO.

Data collection

The following data were collected from all eligible subjects: demographic, perinatal and neonatal data including maternal age, mode of delivery, the presence of prolonged premature rupture of membranes, chorioamnionitis, diabetes, hypertension and use of antenatal steroids, GA, birth weight, gender, respiratory support, duration of total parenteral nutrition, and length of hospital stay.

NTproBNP measurement

In our neonatal intensive care unit, all VLBW infants routinely get complete blood count (CBC) every 2 weeks. After CBC was performed, the left-over blood samples were stored in the laboratory at 4 °C. The collected left-over blood samples were centrifuged at 1000×*g* for 15 min and plasma (125–150 µL) was collected and immediately stored at −80 °C for future measurement of NTproBNP levels. NTproBNP levels were measured by using VITROS NTproBNP reagent pack and VITROS NTproBNP calibrators on the VITROS 5600 ECi/ECiQ Immunodiagnostic Systems. Approximately 40–50 µL of plasma was required to measure NTproBNP. We collected these samples from all the subjects at 28, 32, and 36 weeks CGA.

Echocardiography

ECHO was performed on all subjects at 28, 32, and 36 weeks CGA by a trained and certified pediatric ECHO technician within 12 h of blood collection. The Phillips iE-33 machine was used to perform all the ECHO's. All ECHOs were interpreted by a pediatric cardiologist who was blinded to the study groups and to the NTproBNP levels. All the data were collected and saved in a secure database.

Statistical analysis

Statistical analyses were carried out using SAS©. Procedures used included nonparametric one-way analysis of variance techniques which utilized Dwass, Steel, Critchlow-Fligner for pairwise two-sided multiple comparisons (DSCF). This nonparametric technique was used to compare continuous variables between the three groups. Multivariate general linear model techniques using Bonferroni methods for multiple comparison were used to control for potentially confounding effects of gestational age and birth weight as well as used for repeated measures analysis of variance. Logistic modeling was used for receiver operator curve (ROC) generation and significant differences between ROC curves was determined by chi-square analysis. Analysis of nominal variables across the three groups was carried out by chi-square tests. The level of statistical significance was arbitrarily set at $p < 0.05$.

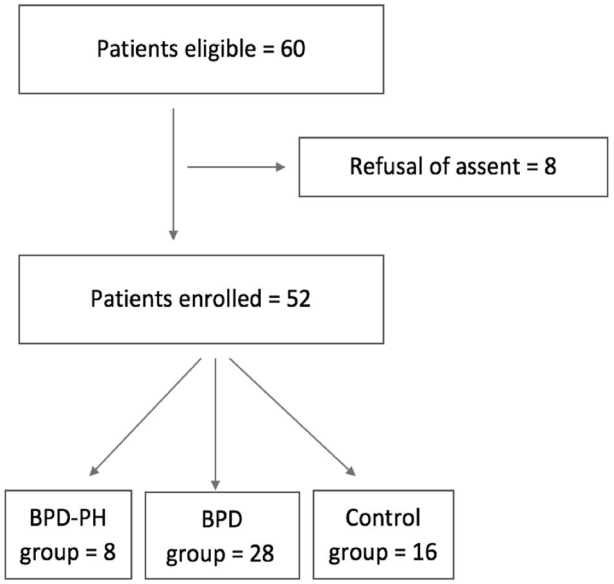

Fig. 1 A figure showing the number of patients in each group

Results

Although 60 infants were eligible for the study, only 52 were enrolled (refusal of assent $n = 8$). Eight infants (15.3%) were in the BPD-PH group, 28 infants (53.8 %) were in the BPD group and 16 infants (30.7%) were in the control group (Fig. 1). Maternal demographic variables such as mean age, mode of delivery, premature prolonged rupture of membranes, chorioamnionitis, diabetes, hypertension, use of magnesium sulfate, or steroids did not significantly differ across the three groups (Table 1). Infant characteristics evaluated included mean birth weight, mean GA, sex, mean days on various modes of oxygen support, mean duration of TPN, and hospital stay (Table 2). Based on nonparametric ANOVA testing, the following significant differences were apparent. The BPD group had a lower birth weight and GA as compared to the control group. Infants in the BPD group spent more days on nasal cannula, SIPAP and conventional mechanical ventilation and had significantly longer duration of mean TPN duration and hospital stay. There was no statistical significance between any of the variables between the BPD_PH group and the control group. We believe this to be because of the lack of power (only eight patients in the BPD-PH group).

There was one infant with borderline PH on ECHO (mean pulmonary artery pressure 24 mmHg) on the ECHO at 28 weeks CGA but no such evidence on subsequent ECHOs and was ultimately enrolled in the BPD group. Eight patients had a PDA out of which two (one in BPD-PH group and one in BPD group) had hemodynamically significant PDAs (>2 mm in diameter). These closed after the administration of indomethacin. The hemodynamically insignificant PDAs in the other patients closed spontaneously. We administer indomethacin for intraventricular hemorrhage prophylaxis to all infants <1000 g which may explain the low incidence of PDA in our cohort. Clinically important outcome data were obtained in all the three groups.

Table 1 Maternal characteristics. Comparisons across groups made by chi-square test

	BPD-PH		BPD		Control	
Mean age (years)	27 ± 2		26.5 ± 1.5		28 ± 1.3	
Mode of delivery (No. of subjects)	Vaginal	CS	Vaginal	CS	Vaginal	CS
	0	8	5	23	4	12
PPROM (No. of subjects)	2		9		8	
Chorioamnionitis (No. of subjects)	1		5		4	
Maternal diabetes (No. of subjects)	1		4		2	
Elevated blood pressure (No. of subjects)	PIH	PE	PIH	PE	PIH	PE
	2	1	9	8	9	6
Magnesium sulfate (No. of subjects)	1		14		11	
Antenatal steroids (No. of subjects)	Total	WB	Total	WB	Total	WB
	6	6	25	19	14	12

PPROM prolonged premature rupture of membranes, *CS* caesarean section, *PIH* pregnancy induced hypertension, *PE* pre-eclampsia, *WB* with benefit

Table 2 Infant characteristics

	BPD-PH		BPD		Control	
Mean birth weight (grams)	979 ± 331		843 ± 225[a]		1061 ± 255	
Mean GA (weeks)	27 ± 2		26.5 ± 1.5[a]		28 ± 1.3	
Sex (No. of subjects)	M	F	M	F	M	F
	7	1	14	14	9	7
Mean days on nasal cannula	12 ± 17		23 ± 15[a]		12 ± 13	
Mean days on CPAP	8 ± 12		15 ± 12[a]		18 ± 13	
Mean days on Sipap	10 ± 16		15 ± 17		3 ± 9	
Mean days on CMV	14 ± 9		13 ± 21[a]		0.56 ± 1	
Mean days on HFOV	3 ± 9		1 ± 3		0 ± 0	
Mean TPN duration (days)	27 ± 11		29 ± 33[a]		14 ± 8	
Mean hospital stay (days)	57 ± 29		84 ± 45[a]		54 ± 24	

Comparisons across groups for nominal variables made by chi-square tests. Comparisons across groups for continuous variable made by one-way analysis of variance using DSCF Methodology for pairwise two-sided multiple comparison analysis

GA gestational age, *CPAP* continuous positive airway pressure, *CMV* conventional mechanical ventilation, *HFOV* high frequency oscillatory ventilation, *TPN* total parenteral nutrition

[a]Significant difference as compared to control group

Table 3 Median NTproBNP values in pg/ml (IQR) at 28, 32, and 36 weeks CGA in the BPD-PH, BPD and control groups

NTproBNP Mean (SD)	BPD-PH	Control	*p* value	BPD	Control	*p* value	BPD-PH	BPD	*p* value
28 weeks	8382 (8567)	500 (613)	<0.01	1200 (1150)	500 (613)	<0.01	8382 (8567)	1200 (1150)	<0.01
32 weeks	9502 (8418)	608 (702)	<0.01	1564 (979)	608 (702)	<0.01	9502 (8418)	1564 (979)	<0.01
36 weeks	9656 (8589)	918 (960)	<0.01	1840 (1892)	918 (960)	<0.01	9656 (8589)	1840 (1892)	<0.01

Statistical significance determined by one-way analysis of variance using DSCF Methodology for pairwise two-sided multiple comparison analysis

The median NTproBNP values (IQR) for infants in the control group were 500 (613), 608 (702) and 918 (960) pg/ml at 28, 32, and 36 weeks CGA, respectively (Table 3). Using nonparametric one-way analysis of variance testing for multiple comparisons, the corresponding values in the BPD-PH group were 8382 (8567), 9502 (8418) and 9656 (8589) pg/ml ($p < 0.05$; compared to control) and in the BPD group were 1200 (1150), 1564 (979) and 1840 (1892) pg/ml ($p < 0.05$; compared to control). Comparison of the BPD-PH with the BPD group showed significantly elevated NTproBNP levels in the BPD-PH group at the same intervals ($p < 0.05$).

In a repeated measures analysis of variance (ANOVA) general linear model that included the GA and NTproBNP levels at 28, 32, and 36 weeks CGA, NTproBNP values among the BPD-PH classified infants remained significantly higher than NTproBNP values in the BPD classified infants. Although the NTproBNP levels for BPD-PH did not change significantly over time, the GA was significantly associated with time demonstrating a small, but significant increase in NTproBNP levels with gestational age. In a similar repeated measures analysis of infants in BPD vs control group adjusting for GA, there were no significant associations with NTproBNP levels at 28 and 32 weeks CGA, however, at 36 weeks CGA, NTproBNP levels were significantly elevated and there was an overall significant interaction between time and GA. ROC curves were generated for NTproBNP levels at 28 weeks CGA which provided a cut-off value of 2329 pg/ml for the detection of BPD-PH (sensitivity 87.5%, specificity 95%) (Fig. 2) and a cut-off value of 578.1 pg/ml for the detection of BPD (sensitivity 89%, specificity 68%) (Fig. 2). Area under the curve contrasts between 28, 32, and 36 weeks did not show any statistically significant differences.

Discussion

Based on our review of literature, this is the first prospective study to perform serial ECHO and measurements of NTproBNP levels at 28, 32, and 36 weeks CGA to determine its utility as a surrogate biomarker for the screening of BPD-PH in preterm infants. We have demonstrated good correlation (high sensitivity and specificity) of NTproBNP cut-off levels at 28 weeks CGA and the development of BPD and BPD-PH at 36 weeks CGA in preterm infants. This study may be the basis of future multi-center larger studies to validate our findings.

BNP is synthesized and secreted mainly by the ventricular myocardium [7]. Within the myocytes, BNP is derived from the precursor preproBNP, which is cleaved to the prohormone proBNP and a signal peptide. Under conditions of sustained ventricular volume and/or pressure overload, proBNP is released into the circulation, where it is cleaved by an unknown protease into the physiologically active hormone BNP, and the inactive metabolite NTproBNP [12]. NTproBNP is a preferred cardiac biomarker as it remains stable at 4 °C for 24 h, has a longer plasma half-life, and higher plasma concentration as compared to BNP [13, 14]. Various studies have demonstrated a correlation between the levels of BNP and NTproBNP with the severity of PH in the adult population [15]. However, studies looking at the utility of using NTproBNP for the screening and diagnosis of BPD-PH in preterm infants are limited.

Our results correlate well with the findings of Montgomery et al. who enrolled PI (<27 weeks GA and birth weight <750 g) to identify NTproBNP as an early surrogate biomarker for BPD-PH in PI [8]. They performed a screening ECHO at 36–38 weeks CGA and measured plasma NTproBNP levels within 1 week. Significantly higher NTproBNP levels were demonstrated in infants in the BPD-PH group. They concluded that NTproBNP may be a cost-effective biomarker for the screening of BPD-PH in preterm infants.

In a retrospective study, Cuna et al. [16] also reported higher BNP levels in premature infants with BPD. On multivariate cox proportional hazard analysis, BNP levels predicted survival independent of age, gender, and BPD severity. Area under the ROC identified a BNP level of 220 pg/ml to have 90% sensitivity and 65% specificity in predicting mortality. They concluded that BNP estimation in preterm infants with BPD-PH may be a useful prognostic biomarker of mortality. The aim of our study was not to correlate NTproBNP levels in predicting mortality. However, we demonstrated high sensitivity and specificity in

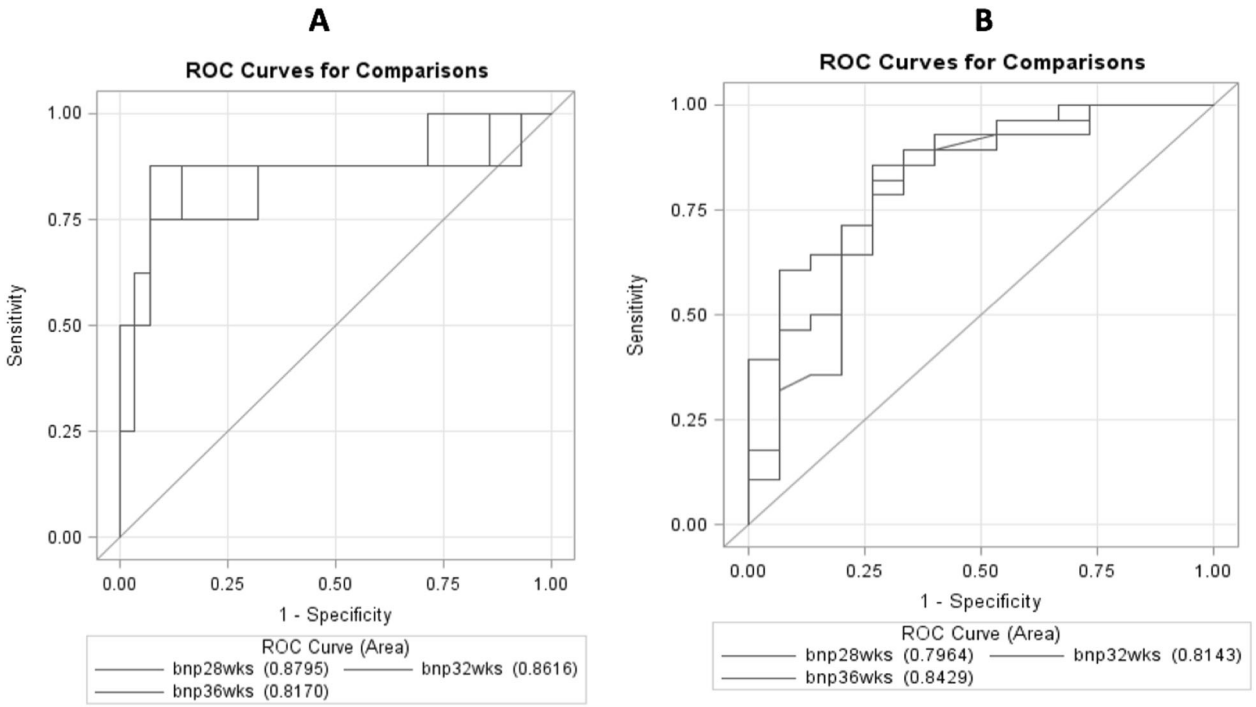

Fig. 2 ROC Curve comparisons: Curve **A**: Control vs BPD with NT proBNP values at 28 weeks, 32 weeks, and 36 weeks. Curve **B**: BPD vs BPD_PH with NTproBNP values at 28 weeks, 32 weeks, and 36 weeks. Area under the curve (AUC) contrast estimation between 28 and 32 weeks; and 28 and 36 weeks demonstrated no significant differences for Curve A or Curve B

predicting development of BPD as well as BPD-PH in preterm infants.

On the other hand, in a more recent retrospective study, Koenig et al. enrolled preterm infants at <32 weeks GA who had an ECHO and plasma levels of BNP and NTproBNP within the first 72 h of life [9]. PH was defined by increased right ventricular (RV) systolic pressure measured by ECHO. They failed to demonstrate any correlation between BNP or NTproBNP and RV systolic pressure in simple and multiple linear regression analysis. The authors concluded that BNP or NTproBNP did not correlate with RV systolic pressure and hence PH in the early postnatal period in preterm infants. However, in this study ECHO was performed to assess persistent PH of newborn and was performed on day 3 of life, which may be too early to detect evidence of BPD-PH.

In contrast to previous studies, we measured serial plasma NTproBNP levels along with ECHO at 28, 32, and 36 weeks CGA. We demonstrated that elevated NTproBNP levels at 28 weeks CGA correlated well with the development of BPD (578.1 pg/ml) and BPD-PH (2329 pg/ml) at 36 weeks CGA. If these results are validated by future studies, it may enable treatment protocols to be modified prior to the development of irreversible pulmonary vascular changes.

Our study demonstrated significantly elevated NTproBNP values in the BPD-PH and BPD group as compared to the control group in serial measurements at 28, 32, and 36 weeks CGA. Comparison between the BPD-PH and BPD group also showed significantly elevated NTproBNP levels in the BPD-PH group during serial measurements. Interestingly, we showed that an NTproBNP value >2329 pg/ml would detect BPD-PH with 87.5% sensitivity and 95% specificity, while a value >578.1 pg/ml would detect BPD with a sensitivity of 89% as early as 28 weeks CGA. If these results are validated by further studies, NTproBNP has the potential of reducing the need for serial ECHO in the management of patients with BPD-PH.

Limitations

The main limitation of our study was the small sample size. Though this is the first study to perform serial measurements of NTproBNP in preterm infants, the results obtained should be validated in larger studies. Another limitation was the use of ECHO for the diagnosis of PH. It could be subjective and operator dependent in the absence of a good tricuspid regurgitant jet. However, in the absence of cardiac catheterization it remains the standard of diagnosis of PH. A third limitation was using NTproBNP as a biomarker of BPD-PH in the absence of defined normal values in preterm infants [5]. Finally, we did not evaluate the effect of treatment (pulmonary vasodilators) on the NTproBNP levels in the patients included in the study. Consideration may be

given to evaluate the trend of NTproBNP levels with therapy in a future study.

Many experts, neonatologists, pediatric pulmonologists, and cardiologists recognize that a sensitive and specific biomarker is needed to improve the screening, diagnosis, and management of BPD-PH in preterm infants. This may help in risk stratification and targeted therapy in those at risk. We believe that the present study is a first step in this direction. Further studies of serial ECHO and NTproBNP measurements are warranted for early screening and eventually the diagnosis of BPD-PH in preterm infants. We suggest using the NTproBNP as a screening tool for the diagnosis of BPD-PH in preterm infants between 28 and 36 weeks CGA.

Conclusions

NTproBNP levels of >2329 pg/ml is a good screening tool to determine the onset of BPD-PH as early as 28 weeks CGA with high sensitivity (87.5%) and specificity (95%). NTproBNP >578.1 pg/ml appears to be 89% sensitive and 68% specific for the prediction of development of BPD as early as 28 weeks CGA. NTproBNP levels at 28 weeks CGA is less specific to predict the development of BPD, although as one might expect, over time, the predictive value appears to improve. At present, it is premature to recommend therapeutic intervention for BPD-PH at 28 weeks CGA based on elevated NTproBNP levels. Further studies are suggested to validate our findings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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