Introduction

The ductus arteriosus serves to bypass the pulmonary artery during fetal life and normally closes within the first 24 hrs of life in term infants [1]. The incidence of a patent ductus arteriosus (PDA) in preterm infants ranges from 20% to 60%, depending on the diagnostic criteria used and population studied [2,3]. Aorticopulmonary shunting at the level of PDA is the main cause of congestive heart failure (CHF) in neonates, which increases ventilator dependence and contributes to chronic lung disease (CLD)[4]. Additionally, feeding difficulties, necrotizing enterocolitis (NEC), intracranial hemorrhage (IVH) may result from the diastolic run-off from the systemic circulation. The ductal flow is proportional to...
the PDA diameter and to imbalance between the systemic and pulmonary vascular resistances [5]. Treatment of clinically significant PDA is either pharmacological by non steroidal anti inflammatory drugs (NSAID) such as Ibuprofen or Indomethacin or by surgical intervention in those who fail pharmaothrapy.

Since physical examination may be unreliable, echocardiography (ECHO) is used to determine the presence and magnitude of PDA shunting. Routine ECHO for the evaluation of PDA in preterm newborns is associated with increased cost, disruption of thermoneutral environment and limited availability in some centers. However, B-type natriuretic peptide (BNP) levels can be rapidly measured at the bedside using a US Food and Drug Administration (FDA)-approved point-of-care testing (POCT) diagnostic device [6]. This assay system has been used in a number of studies of PDA in the pediatric population.

Although mean BNPs are higher in infants with PDA, no consensus exists as to the actual BNP level that should be considered as clinically significant. The levels reported to be significant range from 70 ng/L [7] to more than 1110 ng/L [8].

Recent reports from several centers suggest that the commonly-used treatment of PDA with non-steroidal anti-inflammatory drugs (NSAIDs) may be futile [9] and that N-terminal pro-BNP may not be clinically useful as a biomarker for PDA [10]. These studies, thus, call into question several widely held beliefs about the role of natriuretic peptide assay and NSAID treatment of PDA.

The aim of this study was to 1) Examine, in a group of 52 pre-term neonates, the relationship between the extent of a PDA (if present) and BNP concentrations measured at the point-of-care (POC) and 2) Determine if changes in PDA size after treatment with NSAID are accompanied by decreases in assayed BNP levels. Another potential outcome of this study is the demonstration of the utility of POCT-assayed BNP as a screening tool for PDA in this group of neonates.

Materials and Methods

Setting and protocol. This is a single center, prospective cohort study conducted at regional referral Neonatal Intensive care unit (NICU) at St Joseph’s Children’s Hospital, Paterson, New Jersey from August 2008 to September 2009. The study was approved by the Institutional Review Board at St Joseph’s Regional Medical Center and all subjects had informed, written consent obtained from their family prior to enrollment.

All babies admitted to the NICU (either directly or transferred in) with a birth weight <1250 gm were included in the study. ECHO and BNP were obtained between Day 3 and Day 7 of life. Babies delivered before 34 weeks and with a clinical suspicion of PDA (murmur, worsening of clinical condition, wide pulse pressure/ bounding pulses) were also included. All infants included in the study had normal renal function. Those with genetic anomalies and congenital heart disease except for PDA and patent foramen ovale were excluded.

Blood samples were collected simultaneously with other blood draws to avoid excessive blood draws specifically for study purposes. BNP measurements along with ECHO were performed between Day 3 and Day 7 of life on all neonates < 1250 gm, and in all neonates with gestational age < 34 weeks with clinical suspicion of PDA. ECHOs were read by one of four Pediatric Cardiologists who were blinded to the BNP results. Results were reported as No, Small, Moderate and Large PDA. PDA was managed according to NICU protocols and their BNP was repeated with follow-up ECHO. The attending neonatologist determined the plan of care for each patient, and BNP levels were not available to the cardiologist or the NICU team.

Measurement of Plasma BNP. Plasma BNP levels were determined at bedside using Triage®
BNP assay (Biosite Diagnostics, San Diego, CA, USA) by NICU nurses who were formally trained in the use of this system. Blood was collected in EDTA-containing microtainers. After addition of 0.25 mL of whole blood to the sample port of the test device, the blood cells were separated from the plasma by a filter. In the Triage® BNP assay, plasma enters a reaction chamber that contains murine polyclonal fluorescence-tagged BNP antibodies. The reaction mixture is incubated for 2 minutes. Capillary action results in migration of the reaction mixture through the diagnostic lane to a zone of immobilized murine monoclonal antibody against the ring structure of BNP, binding the BNP fluorescent antibody complex. The unbound fluorescent antibodies were washed away by excess plasma. The Triage BNP device quantifies the fluorescence intensity of the BNP assay zone using an internal calibration curve. The assay required approximately 15 minutes.

ECHO measurements. ECHO measurements were performed using Accuson Sequoia instruments (Siemens AG, Mountain View, CA, USA) using 10v4 and 8v3 transducers. Detection of PDA was performed using 2-dimensional imaging, color flow mapping, and spectral Doppler interrogation from parasternal long and short axis and supra-sternal views. Primary assessment of PDA size was performed taking into consideration ductal diameter at the pulmonary side, absolute size of the PDA in comparison to the branch pulmonary arteries, as described by Ramos et al. [11]. Secondary considerations were length of color flow jet into the main pulmonary artery, presence of diastolic reversal of flow in the descending aorta, and the presence of left atrial and ventricular dilatation including left atrial/aortic ratio. Considering the above parameters, the PDA size, if present, was qualitatively determined as small, moderate or large.

Statistical analysis. Continuous variables were tested for normality using the D’Agostino-pearson omnibus normality test. Invariably, the deviations from normality observed were of a magnitude that suggested the application of nonparametric methods. Thus, differences in continuous variables among groups were ascertained by application of the Kruskal-Wallis test followed by Dunn’s test if the two-tailed p-value for the overall comparison was < 0.05, the level of \( \alpha \) used in this study. Non-parametric trend analysis was performed using Cusick’s test for trends [12]. For two group comparisons of pre vs. post treatment BNP concentration, the Wilcoxon signed rank test was used. Decision levels were determined using receiver operator characteristic (ROC) curve analysis. Prism® software (GraphPad Corp., San Diego, CA, USA) was used for all analyses except Cusick’s test which was computed manually and Cohen’s \( f \), which was computed using G*Power v.3.1 [13].

Results

Upon presentation, 24 subjects had no evidence of a PDA. Twenty-eight had a PDA of which 11 were classified as Small, 6 as Moderate and 11 as Large. The corresponding values of BNP are shown in Figure 1. Overall, we observed a highly significant difference among the groups (\( p < 0.0001 \)). The subjects with no PDA had median (and IQR) BNP concentrations of 23.6 ng/L (13.1 to 32.8). These were significantly different (\( p< 0.05 \)) than those with small PDA for which BNP was 62.9 ng/L (53.6 to 82.1), those with moderate PDA for which BNP was 284 ng/L (204 to 622.0) and those with PDA classified as Large, for which the BNP levels were 2410 ng/L (420 to 2770). Although the three groups of subjects with PDA were not significantly different from one another, the clearly observed groupwise differences are reflected in the results of the trend analysis, for which \( p<0.0001 \). The effect size was estimated from the value of Cohen’s \( f = 0.45 \), suggesting a strong effect.

To establish a BNP cut-off for treatment, we evaluated the decision level from ROC curve provided in Figure 2, which yielded a cut-off of 123 ng/L as a differentiating point between treat and no treat options. However the study did not require an attending neonatologist
to treat on the basis of the cut-offs obtained and, in fact, one neonate with a BNP value on presentation of 111 ng/L was included in the treatment group of 12 subjects. The BNP levels, before and after treatment for these subjects is provided in Figure 3. After treatment, the mean BNP levels decreased from 469 ng/l (233 to 2650 ng/L) to 48.5 ng/L (12.4 to 118 ng/L), a highly significant (p < 0.0005) decrease Figure 3). We should note that these data are for 12 subjects who were treated upon initial presentation. The clinical course for seven of these subjects was complicated and details of follow-up are provided in Table 1, which also includes another subject (subject 6 in Table 1) who was not treated initially but whose condition eventually deteriorated. We should note that the subjects who were not included in Table 1 demonstrated PDA closure upon initial treatment with ibuprofen, i.e., they experienced an uncomplicated course. Most BNP values were generally compatible with the ECHO findings. These subjects’ data are provided in Table 1.

**Discussion**

Respiratory distress in preterm neonates is an important and common symptom and is a marker of multiple significant underlying pathophysiological conditions. Adequate and appropriate measures can prevent significant morbidity. PDA is a frequent, important and treatable cause of respiratory distress in neonates especially pre-term neonates. It is also a risk factor for conditions such as NEC, IVH, and CLD. Rapid and timely determination of hemodynamically significant PDA (hs PDA) is extremely important, but is often difficult.

ECHO is the current gold standard for the diagnosis of PDA and post-treatment follow-up. Several markers of hs PDA have been identified including ratio of left atrial /aortic root (LA/Ao), ductal size and reversed diastolic flow of the descending aorta [13,14], but even with these typical ECHO features, hemodynamic significance and the course of PDA cannot be reliably anticipated. Performing ECHO on
Bedside testing for BNP in neonates with PDA

Newborns has its own limitations and ECHO facilities are generally not available on a twenty-four hour basis in all NICUs. A simple blood test that could easily, rapidly and accurately help in diagnosing hemodynamically significant PDA (hsPDA) would be of great use especially in low birth weight infants. However, currently no accepted blood test is available to aid in the diagnosis and management of PDA in preterm neonates.

BNP is one of the families of structurally similar peptide hormones that also include atrial natriuretic peptide and C –type natriuretic peptide. BNP was first identified (in 1988) in the brain of piglets, but was later found to be secreted predominantly from the cardiac ventricles [15]. It is secreted as Pro-BNP, the inactive precursor that is cleaved into BNP, the active component, and N-terminal- pro- BNP (NTpBNP), an inactive byproduct. The half-life of BNP is 20 min. and of NtpBNP is 60 min [16].

The table below shows the observations in seven subjects with complicated PDA. ECHO findings, BNP levels and therapy. Patients whose initial course of treatment was uncomplicated were not included in this tabulation. The number of observations of the subject neonates is provided across the top of the table, with day of observation below along the indicated row heading.

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Table 1. Observations in seven subjects with complicated PDA: ECHO findings, BNP levels and therapy. Patients whose initial course of treatment was uncomplicated were not included in this tabulation. The number of observations of the subject neonates is provided across the top of the table, with day of observation below along the indicated row heading.

BNP is secreted in response to increased wall stress. This stress may be ventricular wall expansion, pressure overload or increased wall tension. It regulates extracellular fluid volume and blood pressure by natriuresis, diuresis, vasodilation and antagonism of renin –angiotensin- aldosterone system and other vasoconstricting neurohormonal systems through a cyclic guanosine monophosphate second messenger [17,18] and has been used in adults to distinguish cardiac from other non cardiac etiologies in the evaluation of dyspnea [19]. BNP is of diagnostic and prognostic value in the assessment of CHF in adults and is used as a bedside screen for CHF [20], to rapidly differentiate between cardiac and pulmonary causes of respiratory difficulty [21] and as a screening tool for ventricular hypertrophy [20] ventricular diastolic dysfunction [22], transplant rejection [23] and risk of sudden death in adult CHF patients [24].

Several characteristics differentiate premature neonatal heart from that of older infants.
The neonatal myocardium has a higher water concentration and a greater proportion of stiff collagen resulting in non-compliant left ventricle and diastolic dysfunction with relatively poor ventricular filling [25], higher ventricular rate can compound this problem. BNP does not cross the placenta; therefore levels are not affected by maternal conditions. Low levels of BNP can be detected in umbilical blood at delivery. There is a physiological surge in BNP owing to transition from fetal to neonatal circulation, this is associated with an increase of pulmonary blood flow as a result of lung expansion and an elevation of systemic vascular resistance leading to an increase in ventricular volume and pressure load which stimulates BNP secretion to >20 times the umbilical blood level shortly after birth, and reaching plateau levels on days 3-4 followed by steady fall to adult levels by 3 months of life suggesting that BNP has a physiologic regulatory role in the cardiovascular hemodynamic changes that occur during the postnatal period [26].

Plasma BNP concentrations are higher in preterm infants than in healthy term infants for the first few days after birth, and presence of PDA was an important determinant of magnitude of BNP [27,28].

The present study demonstrates that plasma BNP levels in premature neonates are a sensitive marker of the hemodynamic influence of PDA. BNP values measured between Day 3-7 of life correlated significantly with ECHO findings and were helpful in identifying preterm neonates with hsPDA that require treatment. These findings were consistent with the previous studies done by Holmstrom et al [27], Flynn et al [6], and Choi M et al. [8].

Point of care, Triage® BNP Assay (requires only 0.25 mL of whole blood with results in 15 min) was used. It was found that a value > 123 ng/L strongly correlated with the presence of hsPDA requiring further intervention.

Also, it was seen that in patients whose PDA had complex clinical course, serial BNP measurements correlated well with ECHO findings. Plasma BNP levels in the hsPDA group who responded to treatment were found to be significantly lower after pharmacologic closure of PDA, whereas those who didn’t respond had mild or no effect on BNP values after a therapeutic trial. Hence we can conclude that BNP values can be used as a clinical tool to monitor the progress of PDA. These findings differ from the recently published findings of a study by Hammerman et al [10] who suggest that N-terminal proBNP (Nt-proBNP) concentrations were not strongly concordant with PDA size. Based on these findings, they suggested that Nt-proBNP may not have adequate sensitivity to detect hsPDA.

Overall in this study, circulating BNP levels were found to correlate fairly well (although clearly not perfectly) with the ECHO assessments of PDA in preterm infants, and changes in BNP levels were found sensitive to represent ductal shunt changes. A cut off BNP level of 123 ng/L may serve as a differentiating point between treat and no treat options. Moreover, serial BNP measurements may be of value in determining the clinical course of PDA in preterm infants and thus may obviate the need of repeated ECHO in managing patients with PDA. We stress the need to recognize that the use of BNP as a biomarker for PDA severity, despite its widespread acceptance, still warrants further investigation.

The Rapid Assay for BNP measurement in blood seems to be a sensitive and specific test for differentiating preterm infants with and without hsPDA in the neonatal intensive care unit. It may serve as a useful adjunct to ECHO in the management of preterm neonates. Furthermore, the reduction in time concomitant with the use of a POCT method, inevitably enhances, the clinician’s decision-making. Although BNP may not replace ECHO in the diagnosis of PDA, it may obviate the need for repeated echocardiography to confirm ductal closure following treatment.
Acknowledgement

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References
