ACTA PÆDIATRICA
PROMOTING CHILD HEALTH

Acta Pædiatrica ISSN 0803–5253

REGULAR ARTICLE

Early N-terminal pro-brain natriuretic peptide measurements predict clinically significant ductus arteriosus in preterm infants

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Keywords
Brain natriuretic peptide, Natriuretic peptides, Patent ductus arteriosus, Preterm

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Received
30 December 2008; accepted 19 March 2009.

DOI:10.1111/j.1651-2227.2009.01315.x

Abstract

We report a blinded, prospective study of the diagnostic utility of N-terminal pro-brain natriuretic peptide (NTproBNP) measurements for predicting clinically significant patent ductus arteriosus (PDA) and assessing closure.

Methods: Plasma NTproBNP was measured during the first week in 100 preterm babies (mean gestation 28.8 ± 2.9 weeks; mean birth weight 1224 ± 512 g). Echocardiography was performed between days 5 and 7 by operators, blinded to NTproBNP concentration.

Results: NTproBNP peaked on days 2 and 3, declined by day 7. Twenty babies, later treated for PDA, had significantly higher NTproBNP levels throughout. Areas under receiver operating characteristic (ROC) curves were 0.896, 0.897 and 0.931 on days 2, 3 and 7, respectively ($p < 0.0001$). A concentration > 2850 pmol/L had diagnostic sensitivity of 90% and specificity of 89% (95% CI: 68, 99; likelihood ratio 8.10). Ductal closure was associated with a fall in mean NTproBNP from 3003 to 839 pmol/L ($p < 0.001$).

Conclusion: N-terminal pro B-type brain natriuretic peptide (NTproBNP) concentrations peaked and then declined in the first week but remained higher in preterm babies whose PDA required treatment. NTproBNP on day 3 predicted whether a neonatal physician blinded to results would treat a PDA. Fall in plasma NTproBNP indicated closure.

INTRODUCTION

Delayed closure of the ductus arteriosus is a frequent consequence of preterm birth, associated with preventable complications such as chronic lung disease (1) and necrotizing enterocolitis (2). Approximately 65% of infants born before 28 weeks of gestation have a patent ductus arteriosus (PDA) (3) in the neonatal period. Assessing the haemodynamic significance of the duct to inform treatment decisions can be problematic. Echocardiography remains the diagnostic gold standard but requires expensive equipment and trained operator assistance, which is not available in all neonatal units. Biochemical markers of myocardial function may offer complementary physiological information. For example, a small study previously performed in our unit showed that elevated concentrations of B type natriuretic peptide (BNP) during early post-natal life predicted the later presence of a PDA requiring treatment (4). Subsequent studies have confirmed this association (5–7).

Cleavage of a peptide pro-hormone produced by cardiac myocytes, pro B-type natriuretic peptide produces an active 32 amino acid peptide (BNP) and a larger fragment N-terminal fragment (N-terminal proBNP, NTproBNP). Both are released into the circulation, though no function has yet been associated with NTproBNP. Although predictive of haemodynamically significant PDA, BNP has a short half-life of about 20 min and is unstable at room temperature. NTproBNP has a longer half-life (120 min) and is extremely stable when collected as plasma, ethylenediaminetetraacetic acid (EDTA) plasma or serum under a range of storage conditions (8). These properties suggest that it could be more suited to routine clinical monitoring than BNP, although it has been unclear how NTproBNP concentrations relate to haemodynamic status in the neonate.

This prospective and blinded study was undertaken to establish the range of NTproBNP concentrations found in babies of 23–34-week gestation, during the first week of life. We also assessed the value of day 3 NTproBNP concentration in predicting whether a clinician provided with clinical and echocardiographic information (but blinded to NTproBNP concentration) would decide to treat a PDA

Abbreviations

NTproBNP, N-terminal pro brain natriuretic peptide; PDA, patent ductus arteriosus; ROC curve, receiver operating characteristic curve; BNP, B type natriuretic peptide; EDTA, ethylenediaminetetraacetic acid; CV, co-efficient of variation; LA/AO ratio, ratio of the left atrium to the aortic root; SD, standard deviation; CI, confidence interval; pmol/l, picomole per litre; p, probability; CPAP, continuous positive airway pressure.

after day 7. Finally, we studied the effect of medical or surgical closure of the PDA on plasma concentration of NTproBNP.

METHODS

Subjects

Between May 2005 and June 2007, we recruited 102 babies, under 34-week gestation, admitted to the tertiary neonatal intensive care unit at St George's Hospital, London. Two babies were later excluded because they were discharged before completion of the study protocol.

Study design

Blood (0.5 mL in tubes containing lithium heparin) was collected, if sampling was clinically indicated, on days 1, 2, 3 and 7. Cord blood samples were taken where possible. An echocardiogram was performed between day 5 and 7 by a single investigator (SR) blinded to NTproBNP concentration. All treatment decisions were made by attending medical staff, blinded to the NTproBNP concentration but aware of the echocardiographic findings and uninfluenced by the baby's participation in the study. If the PDA was medically or surgically treated, a blood sample was taken before and after treatment to assess the effect of closure. A further echocardiogram was also performed after treatment to assess duct patency.

Laboratory analysis

Samples were transported to the laboratory at room temperature, together with routine samples. On arrival in the laboratory, they were centrifuged at 2000 *g* for 6 min and the supernatant plasma collected. NTproBNP was measured in plasma by electrochemiluminiscent immunoassay, using the Roche Elecsys 2010 analyser (Roche Diagnostics, Burgess Hill, UK). The analytical range was 0.6–4130 pmol/L and the co-efficient of variation (CV) for the assay was 8% at 12.1 pmol/L, 6.4% at 45 pmol/L and 6.9% at 476.6 pmol/L. All NTproBNP results were withheld by the laboratory until close of recruitment in June 2007 to ensure that clinical investigators were blinded to the NTproBNP results.

Echocardiography

An echocardiogram was performed between day 5 and 7 by a single investigator (SR) using a 7 MHz probe (Hewlett Packard, Palo Alto, CA, USA). Ratio of the left atrium to the aortic root (LA/AO ratio) and duct diameter in mm were measured. Structural congenital abnormalities were excluded. The duct was considered haemodynamically significant if the LA/AO ratio exceeded 1.5 and the duct diameter was greater than 1.5 mm (4,9,10). All echocardiograms were independently reviewed by a second investigator (JR). Both echocardiographers were blinded to NTproBNP concentrations.

Ethics

The Wandsworth local research ethics committee approved this study. Written parental consent was obtained in all cases.

Table 1 Clinical characteristics of the babies studied (n = 100) and babies with complete data sets (n = 56)

	Babies studied (n = 100)	Babies with complete data sets (n = 56)
Mean birth weight (SD)	1224 g (512 g)	1162 g (482 g)
Mean gestational age at birth (SD)	28.8 weeks (2.9 weeks)	28.2 weeks (2.8 weeks)
Number of singletons (%)	88 (88)	41 (73)
Number of multiple births (%)	12 (12)	15 (27)
Number of babies mechanically ventilated at birth (%)	41 (41)	31 (55)
Number of babies on CPAP at birth (%)	26 (26)	13 (23)
Number of babies spontaneously breathing in air (%)	33 (33)	12 (22)

Values are expressed as numbers (%) of infants or mean (SD).

Statistical analysis

Categorical data are presented as numbers (%) and continuous data as median (range) or mean (SD), where applicable. Dot plots of NTproBNP values were log transformed to base 10 to aid normality. We used the *t*-test (for unmatched groups) and paired *t*-test (for paired data) to compare continuous variables. Receiver operating characteristic (ROC) curves (11) were generated to estimate the confidence with which NTproBNP concentration at each time point discriminated between babies who would or would not require treatment. Data were analysed and graphs generated using Prism 5 for Windows (Version 5.01; GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Table 1 summarizes the clinical characteristics of the study cohort, and Figure 1 provides information about completeness of data collection. The relatively low proportion of

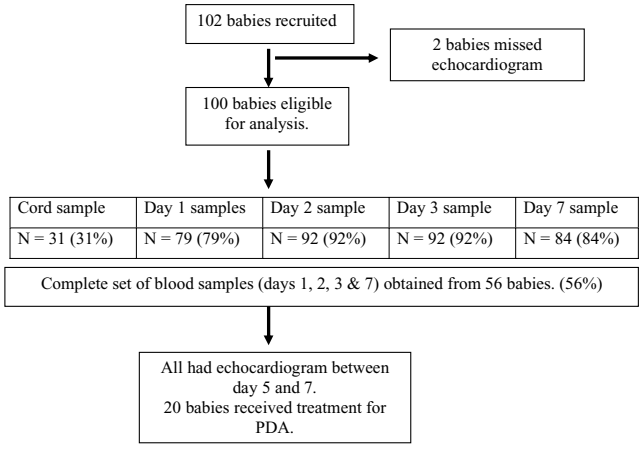

Figure 1 Diagram showing proportions of babies recruited, for whom data were available. In order to avoid bias associated with mixing longitudinal and cross-sectional data, analysis of predictive value was performed only on those babies (n = 56) with complete set of blood samples.

Table 2 Median (interquartile range) of NTproBNP values in pmol/L

Total babies = 100	Babies not treated for PDA (n = 80)	Babies treated for PDA (n = 20)	Total number of successful samples
Cord	749 (204–1685); N = 21	952 (337–1387); N = 10	31
Day 1	918 (515–1983); N = 59	2402 (1593–3898); N = 20	79
Day 2	1206 (657–2211); N = 72	6952 (3134–10135); N = 20	92
Day 3	599 (301–1963); N = 72	6830 (3193–11013); N = 20	92
Day 7	144 (91–295); N = 64	1518 (749–2873); N = 20	84

Median and interquartile range of NTproBNP values of each group at different time periods from all the 100 babies. The column on the right shows the number of successful samples from the above 100 babies at different time points. Babies with haemodynamically significant PDA (LA/AO >1.5; duct diameter >1.5 mm) on echocardiogram were treated by independent clinicians blinded to NTproBNP concentrations, based on clinical and echocardiographic assessment.

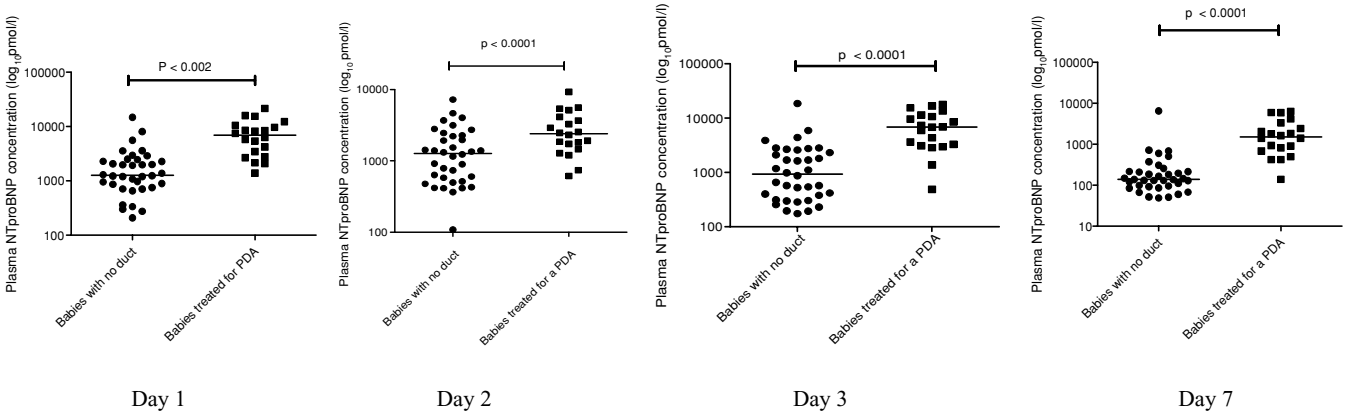

Figure 2 Dot plots showing plasma NTproBNP concentration (log_{10} pmol/L) in treated and untreated babies, complete data sets only (n = 56). Each point represents plasma NTproBNP concentrations log transformed to base 10; the horizontal line represents the median value. Babies who required treatment to close the PDA based on clinical and echocardiographic parameters demonstrated a significantly higher median plasma NTproBNP concentration on each day (Student's *t*-test for unpaired samples).

day 1 samples (79%) reflects a need to allow parents sufficient time to consider information about the study and consent to participation. Thereafter, insufficient sample volume, sample loss and absence of clinical indication for blood sampling were the principal reasons for missing data. Altogether complete post-natal data sets (day 1, day 2, day 3, and day 7) were obtained for 56 babies. Twenty of the 56 babies developed a PDA requiring treatment; some, more than once. Amongst these 20 babies, NTproBNP concentration was measured before and after 28 treatment episodes.

Table 2 provides the median and interquartile range of values for samples collected from the entire cohort of 100 babies at different time points. Plasma NTproBNP concentration was highest on days 1 and 2 and fell on day 7 in those babies who did not require PDA treatment. In babies who later required treatment for a PDA, the NTproBNP concentration was highest on days 2 and 3. We considered it likely that bias would arise through analysis of data from all 100 babies; for example, data missing because there was no clinical indication for sampling would remove the contribution of babies no longer requiring intensive care. To avoid such mixing of longitudinal and cross-sectional data, we repeated our analysis using only data from the cohort of 56 babies from whom complete data were collected between day 1 and day 7 (Fig. 2). A similar trend was observed in this subset: babies who required treatment to close the PDA, showed significantly higher median plasma NTproBNP concentration at all stages (unpaired *t*-test, $p < 0.05$, n = 56).

ROC curves were generated using data from the subset of 56 babies, for whom we had complete data. The area under the ROC curve was relatively consistent between days: 0.896 (95% CI 0.814–0.977), 0.897 (95% CI 0.806–0.988) and 0.931(95% CI 0.859–1.003) on days, 2, 3 and 7, respectively. Thus, on 90–93% of occasions, any randomly chosen baby from the PDA-treated group would be expected to have an NTproBNP value higher than a randomly selected untreated baby.

Figure 3 illustrates the changes in NTproBNP concentrations associated with medical or surgical treatment of the PDA. Twenty-eight pre- and post-treatment comparisons of plasma NTproBNP concentrations were made, as some of the 20 babies were treated more than once. Four babies required surgical ligation. When the PDA closed completely following medical or surgical treatment, mean NTproBNP fell from 3003 pmol/L to 839 pmol/L ($p < 0.0001$, n = 17; Fig. 3a). The variability in response among those whose duct failed to close was explored further. When the duct closed only partially on treatment, mean NTproBNP

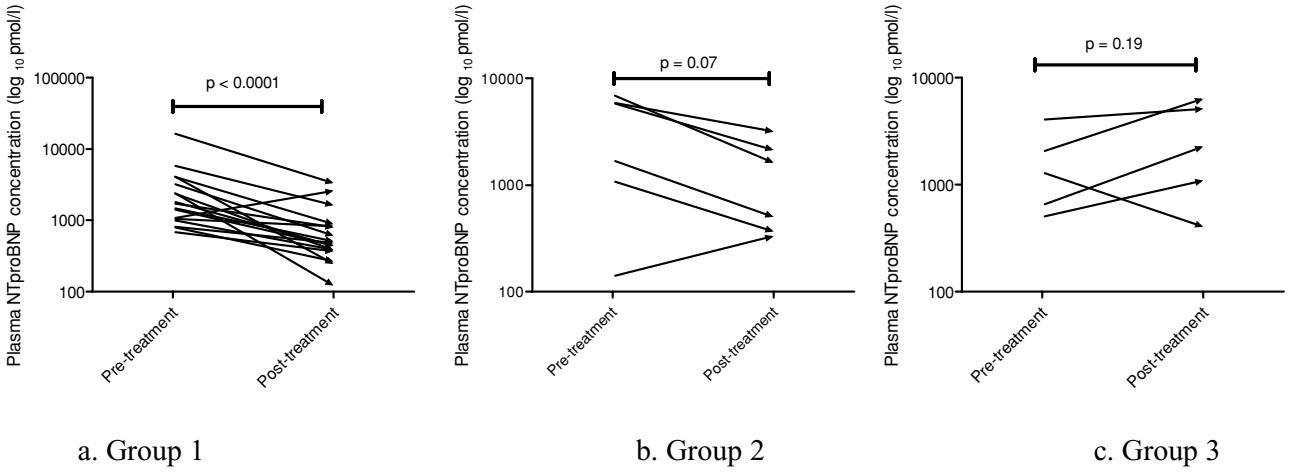

Figure 3 (a)–(c) Change in plasma NTproBNP concentration (log_{10} pmol/L) on treatment of the duct. The lines link paired pre- and post-treatment values in three groups of babies. 'Group 1' were babies in whom the duct closed (medical treatment n = 13; surgical ligation n = 4). In this group, there was a statistically significant fall in NTproBNP concentration ($p < 0.0001$, n = 17, Student's *t*-test for paired samples). Group 2 was babies in whom the PDA partially closed, the residual duct being considered haemodynamically insignificant and subsequently closing spontaneously. In this group, the fall in NTproBNP concentration was not statistically significant ($p = 0.07$, n = 6, paired *t*-test). Group 3 was babies in whom treatment had no effect; in this group, there was no significant fall in NTproBNP concentration ($p = 0.19$, n = 5, paired *t*-test).

Table 3 Relationship between plasma NTproBNP concentration on the second or third day of life and later treatment of a PDA in preterm babies

Reference	Gestation at recruitment (weeks)	N infants/n PDA	Age (d) at sampling	Area under ROC (95% CIs)	Threshold NTproBNP (pmol/L)	Percentage Sensitivity (95% CI)	Percentage Specificity (95% CI)
21	<30	48/25	3	0.866 (0.763–0.969)	>5000	70	87
22	<34	49/18	3	0.978 (0.930–1.026)	>1347	100	95
23	<33	35/12	2	0.964 (0.906–1.022)	>1203	100	91
Present study	<34	56/20	3	0.897 (0.806–0.988)	>1280	95 (75–100)	58 (41–74)
					>2850	90 (68–99)	89 (74–97)
					>5160	60 (36–81)	95 (81–99)

Data shown from this study and others identified in the literature.

N gives the total number of infants recruited and n the number later treated for PDA.

ROC = Receiver Operating Characteristics curve (see text).

El Khuffash et al do not give details of assay but others used the Roche Elecsys assay.

NTproBNP values were converted to pmol/L using a conversion factor of 8.457 pg/mL = 1 pmol/L. The threshold value refers to the NTproBNP concentration on which sensitivity and specificity estimates were based.

concentration fell, though the change was not statistically significant (paired *t*-test, $p = 0.07$, n = 6; Fig. 3b). By contrast, the plasma concentration of NTproBNP rose in four of the five babies in whom the duct failed to close at all, and there was no change in the mean value (paired *t*-test, $p = 0.19$; Fig. 3c).

An NTproBNP concentration greater than 2850 pmol/L on day 3 predicted, with a sensitivity of 90% and specificity of 89% (95% CI 68–99), which babies would have a PDA, judged clinically significant at the end of first week (Table 3). A plasma-NTproBNP concentration exceeding this value was associated with eight-fold greater risk of a PDA that would later need treatment (likelihood ratio 8.10). The positive predictive value was 82%, and the negative predictive value, 90%.

DISCUSSION

In this prospective study, we have shown that early plasma-NTproBNP concentrations in babies under 34-week gestation predict the presence of a PDA that an independent clinician, blinded to NTproBNP concentration, will later decide to treat. The fall in NTproBNP concentration associated with closure was a useful indicator of treatment effect.

PDA is common in babies such as those we studied. Deciding whether or not to treat can be problematic for the physician (12). No single haemodynamic index reliably predicts the risk of PDA-associated morbidity, yet intervention is not without risk. In our unit, the attending physician makes a judgment based on clinical and echocardiographic assessment. By blinding all echocardiographers, attending clinicians and clinical investigators to NTproBNP

concentration, we have shown how NTproBNP concentration can predict the outcome of this process in an unbiased manner. We set out to correlate changes in NTproBNP purely with the decision to intervene and not with echocardiographic measurements. Thus, our study design and analysis could be described as pragmatic rather than explanatory (13). We also followed routine procedures for clinical sampling, specimen transit and handling. Thus, our findings should be generalizable, provided that the same assay procedure is adopted.

A recent systematic review of 19 adult studies (14) concluded that brain natriuretic peptide (BNP) is a strong prognostic indicator of heart failure in asymptomatic patients and those at all stages of disease. In children, it is valuable in assessing the severity of congenital heart disease (15). Holmstrom et al. (16) suggested that the major determinant of plasma-BNP concentrations in preterm infants is the magnitude of shunting through a PDA. Several studies have subsequently confirmed a relationship between BNP concentration in the first week of post-natal life and the presence of haemodynamically significant PDA (4,7,17). Serial BNP measurement has also been used to monitor treatment, helping to shorten duration of therapy (18). Czernik et al., in a blinded prospective study of similar design to ours, recently underscored the predictive value of early BNP measurement in infants under 28-week gestation (19). The value of NTproBNP concentrations has been less widely studied despite the fact that its superior stability in plasma offers significant practical advantages. Czernik et al. expressed a preference for using BNP because it is the active hormone, unlike NTproBNP, which is the inactive remnant of pro-hormone cleavage (19). While cleavage produces equimolar amounts of both, molar concentrations in plasma are not easily comparable because BNP and NTproBNP are differentially cleared (20). There is, consequently, a need to establish separately the utility of NTproBNP in prediction and monitoring of PDA.

Three other studies have assessed the value of early NTproBNP concentration for early prediction of PDA (21–23). These findings are summarized with ours in Table 3. How might they complement echocardiographic assessment in clinical decision making? Overall, the data suggest that a preterm baby with a NTproBNP concentration below 1350 pmol/L on the third day of life is very unlikely to require treatment for a PDA. The specificity of this threshold is, however, more variable, possibly, as a consequence of variation in both clinical management and intervention criteria. Echocardiographic investigation therefore provides useful complementary haemodynamic information in babies where concentration exceeds 1350 pmol/L. The exact place of NTproBNP concentration in the management of ductal disease can only be determined by conducting trials capable of quantifying the outcomes associated with treatment (12) guided by NTproBNP monitoring.

We observed a statistically significant fall in the mean NTproBNP concentration with therapeutic PDA closure. NTproBNP concentration also tended to fall when the PDA did not close but became haemodynamically insignificant, later closing spontaneously. However, the change observed in this group was not statistically significant. There was no change in mean NTproBNP concentration when treatment was ineffective. Indeed, it actually rose in four of the five cases. Thus, we observed graded change in NTproBNP concentration depending on degree of closure. This suggests that it could be as useful as serial BNP measurement for monitoring therapeutic closure (18).

In summary, we found that plasma-NTproBNP concentration early in the first week of life predicts the later presence of a PDA requiring treatment. The fall in NTproBNP concentration associated with treatment is an indicator of successful closure.

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