Plasma Natriuretic Peptides As Predictors of Hemodynamically Significant Patent Ductus Arteriosus in Preterm Neonates

Amr M. Zoair, Nabil M. El-Esawy, Hamed M. El-Sharkawy, Ahmed M. Abd El-Raziek, Mohamed E. El-Seteeha1 and Amal S. El-Bendary2
From the departments of Pediatrics, Cardiology 1 and Clinical Pathology,2 Faculty of Medicine, Tanta University

Abstract:
The objective of this study was to determine whether the plasma levels of natriuretic peptides in preterm infants with patent ductus arteriosus (PDA) are predictors of the hemodynamic significance of the PDA shunt, and to correlate them with the clinical and echocardiographic assessment.

Fifty preterm neonates, with a mean gestational age of 29.4 wk and weighing less than 1500 g, were enrolled in the study. Based on the clinical and echocardiographic findings, the hemodynamic influence of PDA shunt was classified as: large (8 infants), moderate (10 infants), small (12 infants) or no PDA (20 infants). Plasma N-terminal atrial natriuretic peptide prohormone (Nt-pro ANP) and brain natriuretic peptide (BNP) were assessed using ELISA kits.

The results showed that plasma levels of Nt-pro ANP and BNP significantly increased with the size of PDA shunt, and when compared to infants without PDA (P<0.05). A value of Nt-pro ANP > 5000 pmol/l predicted a hemodynamically significant PDA with a sensitivity of 97% and a specificity of 90%, whereas a value of BNP > 25 pmol/l had a sensitivity of 87% and a specificity of 75%. Using echocardiographic left atrial/aortic root ratio (LA: Ao ratio) of 1.5 as a cut off gave a sensitivity of 80% and a specificity of 95%. There were significant positive correlations between these studied parameters (P<0.01).

Conclusion: Plasma natriuretic peptides (Nt-pro ANP and BNP) can be used as predictors of the hemodynamic significance of PDA in preterm neonates, and their measurement may be regarded as complementary to echocardiography in the assessment of PDA shunt and institution of appropriate treatment. Nt-pro ANP is more sensitive and specific predictor than BNP.

Introduction:
Persistent patency of the ductus arteriosus is an important complication of prematurity, and represents an important risk factor for adverse outcome.1 Symptoms attributable to a patent ductus arteriosus (PDA) can be demonstrated in 20-40% of infants with a birth weight below 1000 g,2 with a higher incidence in neonates less than 30 weeks gestation with respiratory distress.3 Clinical signs of PDA can be unreliable, and the clinical diagnosis of a hemodynamically significant PDA lacks accuracy, particularly in the early postnatal days.4,5 Both false negative and false positive clinical findings are common in the first postnatal week.6 Clinical pointers such as increased arterial pulse pressure have been shown not to differ in preterms with PDA.7 As a result, echocardiography has become an important non-invasive method for correct diagnosis to confirm ductal patency.8,9 The most accurate echocardiographic method for diagnosing PDA is direct imaging with color flow and pulsed wave Doppler analysis of the shunt.10,11 These techniques allow assessment of the internal diameter of the PDA and the direction of the shunt.12 Treatment options of PDA in preterm infants include surgical ligation and prophylactic; early (2-4 days after birth) or late (7-10 days after birth) pharmacological treatment with indomethacin.13

The hemodynamic effect of a PDA may, however, be difficult to determine even with echocardiography, and the course cannot reliably be anticipated.12 Natriuretic peptides are rapidly responding cardiac hormones, serving to protect against fluid overload as they inhibit the renin-angiotensin-aldosterone system and have vasodilating, diuretic and natriuretic actions.14 So, high levels of these hormones are present in the plasma of patients with heart failure.15,16 Atrial natriuretic peptides (ANPs) are stored principally in atrial myocytes, and the secretion is mainly determined by increases in transmural atrial pressure with stretch of the left or right atrial wall.17,18 Ventricular tissue normally produces small amounts of ANP, but the production is increased in failing ventricles.19 Moreover, abundant amounts of ANP are present in the myocardium of fetuses and neonates.20 The N-terminal end (1-98) of the ANP prohormone (Nt-pro ANP) is co-secreted with the biologically active ANP, but the circulating levels are higher, more stable and easily measurable.21 So, its measurement...
is regarded as a simple and reliable method to estimate atrial pressure in heart failure and other cardiac diseases as in children with congenital heart disease.22,23 Brain natriuretic peptide (BNP) is secreted by cardiac ventricular tissue to a larger extent than ANP, probably as a response to ventricular stretching.24

The aim of this study was to determine whether the plasma levels of natriuretic peptides (Nt-pro ANP & BNP) in preterm neonates with PDA are predictors of the hemodynamic significance of a PDA, and to correlate their levels with clinical and echocardiographic assessment of PDA shunt.

Subjects and Methods:

The present study was conducted in the Neonatal Unit, Pediatric Department, Tanta University Hospital; in the period from June 2003 to September 2004. Fifty (50) preterm neonates with gestational ages between 27 and 32 weeks and weighing less than 1500 g, were enrolled in the study (on the 4th day of life and re-examined at 7 days of age). Exclusion criteria were cardiac, renal or multiple malformations, and also complicated neonatal course as cerebral hemorrhage, severe respiratory distress syndrome, necrotizing enterocolitis or sepsis. Complete obstetric history was taken and full examination was performed for every infant at the time of admission and time of inclusion in the study. The blood samples (taken on the 4th day of life) were analyzed after completion of the clinical part of the study, and thus did not affect the clinical or echocardiographic assessments.

Clinical assessment:

Clinical assessment of PDA shunt was performed before the echocardiographic examination. The assessment included: type (absent, systolic or continuous) and intensity (degree I-III) of cardiac murmur, foot pulse (not palpable, palpable or bounding) and precordial movements (normal, active or hyperactive).

Echocardiographic assessment:

Echocardiographic assessment of PDA shunt was designed to fit in a clinical setting with critically ill premature infants. All examinations were performed by the same cardiologist (using HP apparatus 5500, USA). The suitable transducer for infants was used (7.5 MHz probe incorporating both pulsed and color flow Doppler). Two dimensional echocardiographic studies were performed for every infant on the 4th and 7th days of life, and when possible on day 28. The procedure for each study was as follows:

- Pulsed and color Doppler assessment of the extension into the pulmonary artery of the ductal signal.
- Direct imaging of the duct (width of ductal color signal, maximal velocity and Doppler flow pattern in the ductal signal).
- Pulsed Doppler recording of post ductal aortic diastolic flow.
- Measurement of left ventricular ejection fraction (EF %), using a standard method.

Based on history, clinical and echocardiographic findings; the hemodynamic influence of ductal shunting was classified as: large, moderate, small or none. A moderate or large PDA was considered (hemodynamically significant).

Blood samples and Natriuretic peptide analyses:

Blood samples were collected through umbilical venous line or venous puncture into chilled plastic vacutainer tubes containing aprotinin and EDTA. The amount of blood was 1.2-2 ml depending on the size of infant. The tubes were immediately placed on ice, centrifuged at 4°C, and within an hour plasma was stored in -70°C for later analysis (within 4 weeks after sampling). Samples were collected within an interval of 3h before and 3h after the echocardiographic assessment.

The following analyses were performed:

1- Atrial natriuretic peptide prohormone; N-terminal (Nt-pro ANP): It was assayed with a competitive Enzyme Immuno Assay, using ELISA kit (Biomedica, Vienna, Austria) which incorporates an immunoaffinity purified sheep antibody specific for Nt-pro ANP. The method is a modification of the radioimmunoassay published by Sundsfjord et al, and has a detection limit of 32 pmol/l.

2- Brain natriuretic peptide (BNP): It was determined by a commercial immunoradiometric assay (Biomedica, Vienna, Austria).

Statistical Analysis:

Statistical analyses for the study were performed with the use of SPSS 8.0 for Windows. Data were expressed as means ± SD. The Student's "t" test was used to compare between two groups. The statistical differences between different groups were tested using Analysis of Variance test (ANOVA). Sensitivity, specificity, and positive and negative predictive values were calculated for estimation of predictive ability. The chosen cut-off points were for Nt-pro ANP 5000 pmol/l, for BNP 25 pmol/l, and for LA/Ao ratio 1.5. The Pearson's correlation coefficient was calculated for bivariate correlations. Data were considered statistically significant at P value < 0.05.
Results:

Table I: Characteristics of the studied preterm neonates

<table>
<thead>
<tr>
<th>Data</th>
<th>Preterm neonates (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td></td>
<td>29.4 ± 1.2</td>
</tr>
<tr>
<td>Sex (M/F ratio)</td>
<td>27/23 (1.17)</td>
</tr>
<tr>
<td>Birth weight (gm): Mean ± SD</td>
<td>1100 ± 200</td>
</tr>
<tr>
<td>Apgar score (1 min)</td>
<td>&gt; 7</td>
</tr>
</tbody>
</table>

Presence of PDA
- No PDA           : 20    (40%)
- Small PDA        : 12    (24%)
- Moderate PDA*  : 10    (20%)
- Large PDA*       :  8    (16%)

*Moderate PDA or large PDA were considered (hemodynamically significant).

Table I shows the characteristic perinatal data of the studied 50 preterm neonates as regards gestational age, sex ratio, birth weight and Apgar score at 1 minute. The table also shows that 20 infants had no PDA (40%), 12 had small PDA (24%), 10 had moderate PDA (20%), and 8 had large PDA (16%).

Table II: Clinical and echocardiographic assessment of studied preterm neonates according to the size of PDA

<table>
<thead>
<tr>
<th>No PDA</th>
<th>Small PDA</th>
<th>Mod. PDA</th>
<th>Large PDA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=20)</td>
<td>(n=12)</td>
<td>(n=10)</td>
<td>(n=8)</td>
<td></td>
</tr>
</tbody>
</table>

Clinical assessment:
- Type of murmur: Absent Systolic Continuous Continuous
- Intensity of murmur: Absent Grade I Grade II Grade III
- Foot pulse: Not palpable Palpable Bounding Bounding
- Precordial movements: Normal Normal Active Hyperactive

Echocardiographic assessment:
- LA: Ao ratio: Mean ± SD
  - No PDA: 1.17±0.18
  - Small PDA: 1.21±0.20
  - Mod. PDA: 1.50±0.20
  - Large PDA: 1.61±0.27
  - P value < 0.05
- Diameter of duct (mm): 0 < 1.5 ≥ 1.5 ≥ 1.5 < 0.05

* Significant.

Table II shows the clinical and echocardiographic data of the studied preterm neonates according to the size of PDA. As regards the echocardiographic assessment, the table shows that the LA: Ao ratio was 1.17±0.18 in infants with no PDA, 1.21±0.20 in those with small PDA, 1.50 ± 0.20 in moderate PDA and 1.61±0.27 in large PDA. The differences between all groups were statistically significant, either analyzed separately or combined (P<0.05). The diameter of the duct (by color flow imaging) was less than 1.5 mm in small PDA and more than or equal to 1.5 mm in moderate and large PDA. The closed duct had no detectable shunt on color flow mapping. The differences between hemodynamically significant PDA groups and other groups were statistically significant (P<0.05).

Table III shows that the plasma Nt-pro ANP levels were significantly higher in infants with PDA as compared to those without PDA (225±80 pmol/l vs 10±2 pmol/l, P<0.001).

Table III: Plasma levels of natriuretic peptides in preterm neonates with and without PDA

<table>
<thead>
<tr>
<th>Plasma natriuretic peptides (pmol/l)</th>
<th>Preterm neonates without PDA (n=20)</th>
<th>Preterm neonates with PDA (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nt-pro ANP : Mean ± SD</td>
<td>4120±250</td>
<td>16280 ± 920</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>3150 – 5120</td>
<td>4205 – 2718</td>
<td></td>
</tr>
<tr>
<td>BNP : Mean ± SD</td>
<td>10 ± 2</td>
<td>225 ± 80</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>6 – 26</td>
<td>15 – 620</td>
<td></td>
</tr>
</tbody>
</table>

* Significant.

Plasma Nt-pro ANP and BNP levels according to the size of PDA (table IV, Fig. 1, 2):

Table IV: Plasma levels of Nt-pro ANP and BNP according to the size of PDA

<table>
<thead>
<tr>
<th>Plasma natriuretic peptide levels (pmol/l)</th>
<th>No PDA (n=20)</th>
<th>Small PDA (n=12)</th>
<th>Mod. PDA (n=10)</th>
<th>Large PDA (n=8)</th>
<th>P value by ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nt-pro ANP: Mean±SD</td>
<td>4120±250</td>
<td>5850±320</td>
<td>16950±480</td>
<td>16730±650</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Range</td>
<td>3150–5120</td>
<td>4205–6720</td>
<td>9158–2830</td>
<td>13147–27164</td>
<td></td>
</tr>
<tr>
<td>BNP: Mean±SD</td>
<td>10±2</td>
<td>29±11</td>
<td>178±66</td>
<td>245±82</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Range</td>
<td>6–28</td>
<td>15–57</td>
<td>53–440</td>
<td>135–620</td>
<td></td>
</tr>
</tbody>
</table>

* Significant.

Fig.1: Plasma Nt-pro ANP levels according to the size of PDA

Fig.2: Plasma BNP levels according to the size of PDA

Table IV and figures 1,2 show that the plasma levels of Nt-pro ANP and BNP significantly increased with the size of the shunt through a PDA from small to
large, and when compared to preterm neonates without PDA (P<0.05).

Table V: Sensitivity, specificity, positive and negative predictive values of plasma natriuretic peptides and LA/Ao ratio as predictors of hemodynamically significant PDA in preterm neonates

<table>
<thead>
<tr>
<th>Cut-off levels</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nt-pro ANP (5000 pmol/l)</td>
<td>97%</td>
<td>90%</td>
<td>94%</td>
<td>95%</td>
</tr>
<tr>
<td>BNP (25 pmol/l)</td>
<td>87%</td>
<td>75%</td>
<td>84%</td>
<td>80%</td>
</tr>
<tr>
<td>LA: Ao ratio (1.5)</td>
<td>80%</td>
<td>95%</td>
<td>96%</td>
<td>76%</td>
</tr>
</tbody>
</table>

Table V shows that a value of Nt-pro ANP above 5000 pmol/l predicted a hemodynamically significant PDA with a sensitivity of 97%, a specificity of 90%, a positive predictive value of 94% and a negative predictive value of 95%. The table also shows that a value of BNP above 25 pmol/l predicted a hemodynamically significant PDA with a sensitivity of 87%, a specificity of 75%, a positive predictive value of 84% and a negative predictive value of 80%. As regards the LA: Ao ratio (by echocardiography), using a cut-off point of 1.5, predicted a hemodynamically significant PDA with a sensitivity of 80%, a specificity of 95%, a positive predictive value of 96% and a negative predictive value of 76%.

Figure 3 shows that there was a significant positive correlation between plasma Nt-pro ANP and LA: Ao ratio (by echocardiography) (r = 0.986, P<0.01), whereas figure 4 shows that there was a significant positive correlation between plasma BNP and LA: Ao ratio (r = 0.991, P<0.01). Also, figure 5 shows a significant positive correlation between plasma levels of Nt-pro ANP and BNP in preterm neonates with PDA (r = 0.970, P<0.01).

Discussion:

It has been recently proven that the plasma natriuretic peptides in healthy neonates show a marked increase during the first days of age, suggesting that ANP and BNP have physiologic roles in the perinatal circulatory change from fetus to neonate, then their levels descend and remain almost constant after the 4th day of life, with higher concentrations in preterm infants, possibly due to physiologic stress, respiratory distress and reduced renal function.

In the present study, the plasma concentrations of Nt-pro ANP and BNP were significantly higher in preterm neonates with PDA as compared to those without PDA. Moreover, the plasma levels of these natriuretic peptides significantly increased with the size of the shunt through PDA from small to large. They were strongly related to the hemodynamic influence of PDA.

A strong relation between plasma ANP and PDA in premature infants was described before by many authors, but without determination of the diagnostic precision of these peptides. On the other hand, our results agree with that of Holmstrom et al., who reported that there was a diagnostic value of the N-terminal fragment of ANP prohormone (Nt-pro ANP) for the hemodynamic assessment of PDA in premature infants. The levels of prohormone are considerably more stable, and the pulsatile pattern of secretion of the peptide will not be manifest by...
assessment of the prohormone because of its longer half-life.\textsuperscript{21,22} It correlates to the severity of cardiac disease\textsuperscript{42} and the extent of left-to-right shunting.\textsuperscript{30} Holmstrom et al.\textsuperscript{23} reported that elevated levels of Nt-pro ANP reflect atrial pressure or volume overload in children with congenital heart disease, resulting in atrial dilatation and/or elevation of pressure, with subsequently increased wall tension and release of peptide.

The results of the present study are also in agreement with that of Puddy et al.,\textsuperscript{43} who reported that elevated BNP concentrations on day 4 after birth were predictive of the presence of PDA in preterm infants at the end of the first week of life.

There are possible causes of cardiac strain in premature infants besides a PDA which may affect circulating natriuretic peptide levels, including increased pulmonary vascular resistance and right ventricular pressure overload but they seem to have little influence on Nt-pro ANP and BNP.\textsuperscript{29,36} Moreover, non-cardiac factors may also affect peptide levels in preterm infants,\textsuperscript{44} including renal impairment,\textsuperscript{37} hydration status,\textsuperscript{35} respiratory distress syndrome,\textsuperscript{36} and the effect of medical therapy with corticosteroids.\textsuperscript{44} However, all these non-cardiac variables have indefinite minor influence on circulating natriuretic peptides as compared to hemodynamic stress factors like PDA.\textsuperscript{35,40,41}

The results of the present study showed that the LA: Ao ratio was a useful echocardiographic measurement in the diagnosis of a hemodynamically significant PDA in preterm infants. Also, using direct color flow imaging of the duct, the diameter of ductal signal significantly increased in large and moderate PDA as compared to small PDA. These results agree with Iyer and Evans,\textsuperscript{25} who reported that the measurement of the LA: Ao ratio is a useful and reasonably accurate test for a hemodynamically significant PDA. It depends on the fact that left to right ductal shunting increases the volume load on the left side of the heart, so the left atrium will dilate relative to the aortic root which will be relatively unaffected by the volume load.

Moreover, our results are in agreement with those of many authors,\textsuperscript{5,6,8-12} who concluded that direct ductal assessment, with measurement of the internal diameter of the duct, using two dimensional and pulsed and color Doppler techniques is clearly the most accurate method for diagnosis of a PDA. On the other hand, clinical diagnosis of a hemodynamically significant PDA lacks accuracy.\textsuperscript{5,12}

As regards the predictive ability, the present study showed that Nt-pro ANP was more sensitive and specific than BNP for prediction of hemodynamically significant PDA in preterm neonates (sensitivity 97% vs 87%, specificity 90% vs 75%, positive predictive value 94% vs 84%, and negative predictive value 95% vs 80%). This is in agreement with Holmstrom et al.\textsuperscript{41} as regards the predictive ability of Nt-pro ANP, but it doesn't agree with them as regards BNP as they reported the same diagnostic value of both peptides. Also, Puddy et al.\textsuperscript{43} reported that BNP was as accurate as ANP in detecting PDA in preterm infants. However, our results showed a significant positive correlation between plasma levels of Nt-pro ANP and BNP in preterm neonates with PDA. Holmstrom et al.\textsuperscript{41} said that no specific differences were identified in the pattern of secretion of Nt-pro ANP and BNP with a close interrelation between them in the plasma of premature infants, whereas Ikemoto et al.\textsuperscript{45} reported a difference between BNP and ANP in the pattern of secretion in preterm neonates.

The present study also showed that using a cut-off point of 1.5, the LA: Ao ratio predicted a hemodynamically significant PDA with a sensitivity of 80%, a specificity of 95%, a positive predictive value of 96% and a negative predictive value of 76%. Moreover, there was a significant positive correlation between the LA: Ao ratio and plasma Nt-pro ANP and BNP. These results are in agreement with Iyer and Evans,\textsuperscript{25} who reported that the accuracy of the LA: Ao ratio was further improved by increasing the cut-off to 1.5. Nineteen out of 21 preterm infants in their study, who needed treatment for a symptomatic duct had LA: Ao ratio of greater than 1.5 at the time of treatment.

Also, Holmstrom et al.\textsuperscript{41} reported that circulating levels of natriuretic peptides correlated well to the echocardiographic and clinical assessment of PDA as echocardiography provided an interpretation of the hemodynamic condition, whereas peptides represented a measurement of the physiologic response to hemodynamic alterations. On the other hand, Puddy et al.\textsuperscript{43} reported that BNP measurements may represent an alternative to echocardiography in the diagnosis of PDA in preterm infants as echocardiographic interpretation may be influenced by equipment and operator skill. Holmstrom and Omland\textsuperscript{46} reported that BNP may provide information not obtained from echocardiography concerning the hemodynamic importance of the PDA shunt in preterm infants.

Moreover, measurements of natriuretic peptides may give supportive guidance in preterm infants with a PDA of uncertain significance, especially if repeated, as repeated measurements might indicate whether changes in the clinical course relate to hemodynamic factors.\textsuperscript{41,47}
Conclusions:
1. Plasma natriuretic peptides (Nt-pro ANP and BNP) can be used as predictors of the hemodynamic significance of PDA in preterm neonates. Nt-pro ANP is more sensitive and specific predictor than BNP as a diagnostic marker in the evaluation of PDA shunt.
2. Measurement of natriuretic peptides should not be regarded as a substitute, but as a complementary tool to echocardiography (especially the LA/Ao ratio) in the hemodynamic assessment of premature infants with PDA.
3. These data would allow timely and appropriate treatment of PDA to be instituted, either medical or surgical intervention.

References: