The role of n terminal - probrain natriuretic peptide in the diagnosis of hemodynamic persistent asrteriosus ductus in premature neonates patient

D Dasrak, M M Djer* and N Advani

Department of Children Health, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
*E-mail: muldjer@yahoo.com

Abstract. Persistent ductus arteriosus is one of the most frequent congenital heart diseases found in infants, mainly in preterms. Echocardiography is the gold standard for the diagnosis of hemodynamically significant patent ductus arteriosus (hs-PDA) in preterm neonates. A few studies have suggested that the use of a simple blood assay to detect N-terminal pro-brain natriuretic peptide (NT-proBNP) may be useful in determining the diagnosis and management of hs-PDA. No such studies have been conducted in Indonesia, although the assay kit and characteristics of the patient (gestational age and chronological age) influence the accuracy of NT-proBNP levels in determining hs-PDA. The aim of this study was to determine the association between the NT-proBNP level and the prevalence of hs-PDA in an Indonesian patient population. A cross-sectional study was conducted at Dr. Cipto Mangunkusumo Hospital. PDA was determined using echocardiography in 49 preterm neonates (gestational age <37 weeks and birthweight <2000 g). Subsequently, these patients were divided into three groups: non-PDA, non-hs-PDA, and hs-PDA. The blood NT-proBNP level was then determined in the non-hsPDA and hs-PDA groups, and between-group differences were compared. Among the 49 neonates, 33 patients had PDA, and 16 of these had hs-PDA. The results revealed a significant association between the NT-proBNP level and hs-PDA (p < 0.001).

1. Introduction
The ductus arteriosus, which connects the pulmonary artery and aorta, is an essential component of the fetal circulation. After birth, the ductus arteriosus usually closes within 48 h. Failure to close within 72 h after birth is referred to as persistent ductus arteriosus or patent ductus arteriosus (PDA). PDA is relatively common, especially in premature infants [1]. Natural closure of the ductus arteriosus depends on gestational age and birth weight. Failure to close was reported in 40% of neonates with birthweight < 2000 g [2]. Although the incidence of PDA in premature infants in Indonesia is not known, according to research in other countries, it ranges from 20% to 60%. In a study of premature infants aged 26–32 weeks, Rakza et al. reported that the incidence of PDA was greater in premature infants with growth retardation as compared to infants of appropriate weight according to their gestational age [3]. Genetics also seems to play a role in the incidence of PDA in premature infants [4]. According to Dagle et al., single nucleotide polymorphisms in the transcription factor activator protein-2 β and tumor necrosis factor receptor-associated factor 1, both of which are involved in the synthesis of prostacyclin, are implicated in PDA [4].
Hemodynamically significant PDA (hs-PDA) occurs in around 20% of premature infants with very low birthweight (VLBW) and gestational age <28 weeks. Comorbidities, such as intraventricular hemorrhage and necrotizing enterocolitis, can occur in premature infants with hs-PDA due to the presence of a left-to-right shunt and lead to increased mortality [5]. However, not all premature infants with hs-PDA require therapy. According to one study, closure of the PDA occurred in the absence of treatment in two-thirds of premature neonates [6].

The early management of hs-PDA in premature infants is intended to improve symptoms of heart failure or hemodynamic derangement, shorten the duration of ventilator usage, and reduce the duration of the hospital stay. At present, a clinical assessment, radiological imaging, and echocardiography are used to diagnose PDA, all of which have various limitations. For example, clinical findings, such as continuous noise and a bounding pulse, are not always present in cases of hs-PDA, especially in VLBW infants placed on a mechanical ventilator. Although echocardiography has become the gold standard for the diagnosis of PDA, the assessment does not reflect rapid dynamic changes in circulation, especially in VLBW infants. At present, there is no consensus on the determination of hs-PDA based on echocardiography criteria. An additional problem with the use of echocardiography is that the ductal diameter in cases of PDA is not always related to the degree of ducal shunting [7].

Brain natriuretic peptide (BNP) is a natriuretic peptide synthesized by cardiac myocytes in the atrium and ventricles in response to volume overload or expansion. ProBNP is the active form of BNP, and N-terminal proBNP (NT-proBNP) is the inactive form. BNP has a half-life of 20 min, and NT-proBNP has a half-life of 90 min. As NT-proBNP does not exhibit circadian variations and remains stable in blood, blood samples can be stored and used in subsequent analyses. Based on these properties, NT-proBNP is considered a better marker than BNP in the assessment of ventricular dysfunction and volume overload in the heart [8,9].

Some previous studies examined the potential role of NT-proBNP as a marker of hs-PDA in premature infants. Ramakrishnan et al. reported that NT-proBNP levels decreased in in the first week but not in premature infants with PDA [9]. They concluded that the NT-proBNP level on the third day post-birth could predict whether treatment was required in premature infants with PDA. Nuntinarumit et al. concluded that the plasma level of NT-proBNP on the second day of birth was a sensitive marker to predict the existence of hs-PDA in premature infants. In these studies, a decrease in NT-proBNP levels indicated that the ducts had closed [9,10]. There have been no studies in Indonesia on the association between NT-proBNP levels and ductal closure in PDA. According to a systematic review by Kulkarni, the diagnostic accuracy of NT-proBNP levels in predicting hs-PDA varied, with the characteristics of the assay and threshold levels, in addition to the characteristics of the patients (gestational age and chronological age), affecting the level of accuracy [11]. Kulkarni, concluded that validation of NT-proBNP was necessary prior to using this marker as a basis to start therapy or assess the therapeutic response in hs-PDA. The aim of the present study was to determine the association between NT-proBNP levels and the prevalence of hs-PDA in an Indonesian patient population. The findings can shed light on the potential utility of NT-proBNP as a diagnostic marker of hs-PDA, thereby aiding health care decision making and services for premature infants with hs-PDA.

2. Materials and Methods

This was an analytical observational cross-sectional study designed to determine NT-proBNP levels in premature infants with PDA. The study was conducted in the integrated Heart Services/Neonatal Intensive Care Unit (NICU) of the National Central General Hospital (Dr. Cipto Mangunkusumo) in Jakarta. The target population was premature infants with PDA who were admitted to the NICU of Dr. Cipto Mangunkusumo Hospital between December 2015 and February 2016.

The inclusion criteria were as follows: gestational period <37 weeks, weight <2000 g, and PDA detected on echocardiography. The exclusion criteria were the presence of heart disease other than PDA, major congenital disease, severe asphyxia and an Apgar score <3 in the first to fifth minute after birth, and sepsis with multiple organ failure. Additional exclusion criteria were necrotizing enterocolitis; renal failure; persistent pulmonary hypertension of the newborn; indomethacin,
paracetamol, or ibuprofen therapy administered prior to the measurement of NT-proBNP levels; and parental refusal for the infant’s participation in the study. Patients were selected for inclusion in the study consecutively at the start of the research. All the parents signed written consent forms after receiving an explanation about the nature of the study. The study was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia.

All premature infants <2000 g underwent a clinical examination and echocardiography after obtaining the consent of the parents. Data on the following were recorded during the clinical examination and initial assessment: gestational age, chronological age, gender, birthweight, Apgar score, vital signs, and SaO2 with oximetry, in addition to the existence of anomalies and comorbidities. All the infants also had a routine blood examination. In addition, the mother’s age at the time of birth and type of delivery were recorded. The echocardiography examination was performed under the supervision of a mentor or child cardiologist consultant. All the examinations were performed using a Philips HD11XE machine, with an s12 transducer in the NICU of Dr. Cipto Mangunkusumo Hospital, Jakarta. The ductal diameter was measured from the parasternal short axis view or suprasternal long axis view on two-dimensional echocardiography, with color flow Doppler imaging. The average speed of flow and diastolic flow velocity of the left pulmonary artery were determined. The ratio of LA/Ao was assessed from the parasternal long axis view using M-Mode. The presence of heart disease other than PDA and persistent pulmonary hypertension of the newborn was recorded. Fifty-one infants fulfilled the criteria and were included in this research. Blood plasma samples for NT-proBNP analysis were obtained from all the patients and sent to the laboratory on the same day. Subsequently, the patients were divided into three groups: non-PDA, non-hs-PDA, and hs-PDA. The data were analyzed using SPSS V20. To compare the hs-PDA and non-hs-PDA groups, the X2 test or Fisher’s exact test was used for categorical variables. For continuous variables, the student’s t-test was used for data with a normal distribution, and the Mann–Whitney U test was used for data that were not normally distributed. P-value less than 0.05 were considered to denote statistical significance in the study.

3. Results and Discussion

3.1 Results

Fifty-one premature infants with a birthweight <2000 g who were treated in the Perinatology Division of Dr. Cipto Mangunkusumo Hospital between December 2015 and February 2016 were included in the study. Of these, two infants were excluded. One patient with PDA was excluded because of the presence of a major anomaly (gastroschisis), and the other was excluded because of the presence of a ventricular septum defect. Table 1 presents the demographic characteristics of the patients included in the study.

| Demographic Characteristics | n = 49 |
|-----------------------------|-------|
| Gender, n (%)               |       |
| Boy                         | 22 (44.9) |
| Girl                        | 27 (55.1) |
| Age, average (SD), (days)   | 2.9 (3.17) |
| Gestational age, average (SD), weeks | 30.98 (3.0) |
| Birthweight, average (SD), g | 1401.12 (350.85) |
| PDA, n (%)                  |       |
| With                        | 33 (67.3) |
| Without                     | 16 (32.7) |

Most patients with PDA, were classified as non-hs-PDA. Among the 11 male patients with PDA, 7 (63.6%) were classified as non-hs-PDA, and 4 (36.4%) were classified as hs-PDA. Among the 22
female patients with PDA, 10 (4.5%) patients were classified as non-hs-PDA, and 12 (54.5%) were classified as hs-PDA (Table 2). The average age of the patients in non-PDA group was 3.75 days. In the non-hs-PDA group, the average age was 1.94 days, and it was 3.06 days in the hs-PDA group. There was no statistically significant difference in the average age in the three groups. The gestational age of the non-PDA group was 31.38 weeks, whereas it was 31.41 weeks and 30.13 weeks in the non-hs-PDA group and hs-PDA group, respectively. No statistically significant difference was found in the average gestational age of the three groups.

Table 2. Demographic characteristics of the patients based on the existence of PDA

| Demographic Characteristics | No PDA (n = 16) | PDA (n = 33) | p-value |
|----------------------------|----------------|-------------|---------|
|                            |                | non-hs-PDA (n = 17) | hs-PDA (n = 16) |        |
| Gender, n (%)              |                |                |         |        |
| Boy                        | 11 (50.0)      | 7 (31.8)       | 4 (18.2) | 0.042  |
| Girl                       | 5 (18.6)       | 10 (37.0)      | 12 (44.4)|        |
| Age (SD), days             | 3.75 (4.54)    | 1.94 (1.35)    | 3.06 (2.79)| 0.128  |
| Gestational age (SD), weeks| 31.38 (3.79)   | 31.41 (2.43)   | 30.13 (2.63)| 0.688  |
| Birthweight (SD), g        | 1451.88 (383.21)| 1500.88 (331.44)| 1244.38 (299.42)| 0.801  |

The average birthweight of the hs-PDA group was 1244.38 g, which was lower than that of the non-PDA group (14561.88 g) and non-hs-PDA group (1500.88 g). Although there was a between-group difference in the birthweights, the finding was not statistically significant (p = 0.801). The average NT-proBNP level in the hs-PDA group was 22783 pg/ml, which was higher than that of the non-hs-PDA group (14404 pg/ml). The difference in the average NT-proBNP level of the hs-PDA group and non-hs-PDA group was statistically significant (p < 0.001) (Table 3).

Table 3. NT-proBNP levels of the patients with non-hs-PDA and hs-PDA (mean±SD)

| NT-proBNP, average (SB) pg/ml | Non-hs-PDA (n = 17) | hs-PDA (n = 16) | p       |
|------------------------------|---------------------|-----------------|---------|
|                              | 14404               | 22783           | < 0.001 |
|                              | (15747)             | (16643)         |         |

3.2 Discussion

In the present study, low birthweight (LBW) was defined as <2000 g, and preterm was defined as gestational age <37 weeks. Thirty-three (67.3%) infants had PDA, of whom 17 (51.5%) had non-hs-PDA and 16 (48.5%) had hs-PDA. We used born weight <2000 g to remove infants with dysmaturity. The incidence of PDA was difficult to determine in the present study, as not all premature infants in the hospital underwent echocardiography. In addition, around 34% of cases of PDA in premature infants close spontaneously two to seven days after birth [12], with closure strongly dependent on the presence or absence of hemodynamic disorders. In this research, echocardiography was performed an average of 2.9 days after the birth of the infants. Although PDA may close spontaneously after this time, we defined PDA closure and hs-PDA based on echocardiography performed. Some previous research used criteria other than echocardiography to confirm the diagnosis of hs-PDA. In the present study, to establish the diagnosis of hs-PDA on echocardiography, we used a ductal diameter of more than 1.4 mm/kg, a ratio of LA/Ao more than 1.4, average speed of flow in the left pulmonary artery of more than 0.42 m/sec, or average speed of flow in the left pulmonary artery when diastolic pressure was more than 0.2 m/sec.

As reported earlier, the incidence of PDA varies and depends on the criteria used to diagnose its presence. According to previous studies, the incidence of PDA in premature infants was around 20%, and the incidence was higher (60%) among infants with gestational age <32 weeks [13,14]. The incidence of PDA in VLBW infants was reported to be around 30%. Research also showed that most infants with birthweights <1000 g had PDA and that spontaneous closure occurred after 2–7 days of
life in around 34% of cases [15]. In the present study, 33 (67.3%) of the 49 patients had PDA, of whom 22 (66.7%) patients were females. In a previous study of 318 infants with gestational age <32 weeks, 100 (31.4%) infants had PDA. In another study of 225 premature neonates, 142 (58.7%) had PDA. In a study conducted in Indonesia, the researchers reported that 32% of premature neonates had PDA [16]. In the present study, there was no significant difference between the groups (non-PDA, non-hs-PDA, and hs-PDA) according to gestational age. In terms of birthweights, those of the three groups differed: 1451.88 g, 1500.88 g, and 1244.38 g in the non-PDA, non-hs-PDA, and hs-PDA groups, respectively. However, the difference was not statistically significant. A previous study of premature infants and VLBW infants reported that gestational age, LBW, and surfactant were risk factors for PDA [17]. Another study concluded that LBW and the existence of hyaline membrane disease were risk factors for PDA in premature infants [18].

NT-proBNP is the last fragment (fragments deals) NH2 prohormone BNP secreted by cardiac myocytes. Increased NT-proBNP occurs because the stimuli power limit hold (stress stimulus) wall of the heart in the form of high-yield strain wall arrhythmia and cardiac filling pressure, caused by various causes. In addition to its use as a biomarker of acute and chronic heart failure, NT-proBNP has also been used to determine dysfunction of the left ventricle in asymptomatic patients with risk factors for cardiovascular disease risk [19]. Previous research confirmed that the value of NT-proBNP as a marker of PDA in premature infants was closely related to hemodynamics, with NT-proBNP levels significantly higher on the second day after birth in infants with hs-PDA as compared to infants with non-hs-PDA [9]. In the present study, the NT-proBNP levels of the patients with non-hs-PDA and hs-PDA were 14404 pg/ml and 22783 pg/ml, respectively. The findings of this study are in accordance with those of previous research, which found that the NT-proBNP value was higher in patients with hs-PDA (24420 ±3 190 pg/ml) as compared to patients with non-hs-PDA (3072 ± 332 pg/ml) [7]. According to Ramakhrisnan [9], the level of NT-proBNP was more than 2850 pg/ml on the third day after birth, and it had sensitivity of 90% and specificity of 89% in determining the existence of hs-PDA. Based on the literature and findings of the present study, NT-proBNP can be considered an important marker of hs-PDA due to its high sensitivity and specificity.

4. Conclusion
Based on the results of this study, it can be concluded that the prevalence of PDA in premature infants <2000 g born at Dr. Cipto Mangunkusumo Hospital number is 67.3%. The NT-proBNP level in patients with hs-PDA was significantly higher than that of non-hs-PDA patients. However, further research with a larger number of patients, as well as studies of the risk factors for hs-PDA in premature infants, is needed.

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