RESEARCH ARTICLE

N-Terminal Pro-B Type Natriuretic Peptide as a Marker of Bronchopulmonary Dysplasia or Death in Very Preterm Neonates: A Cohort Study

Anna Sellmer1,2,3*, Vibeke Elisabeth Hjortdal4, Jesper Vandborg Bjerre1, Michael Rahbek Schmidt5, Patrick J. McNamara6, Bodil Hammer Bech7, Tine Brink Henriksen1,2

1 Department of Pediatrics, Aarhus University Hospital, Aarhus, Denmark, 2 Perinatal Epidemiology Research Unit, Aarhus University, Aarhus, Denmark, 3 Department of Pediatrics, Herning Regional Hospital, Herning, Denmark, 4 Department of Cardiothoracic surgery, Aarhus University Hospital, Aarhus, Denmark, 5 Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark, 6 Division of Neonatology, Hospital for Sick Children, Toronto, Canada, 7 Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus, Denmark

* anna.sellmer@clin.au.dk

Abstract

Background
Bronchopulmonary dysplasia (BPD) is a serious complication of preterm birth. Plasma N-terminal pro-B type natriuretic peptide (NT-proBNP) has been suggested as a marker that may predict BPD within a few days after birth.

Objectives
To investigate the association between NT-proBNP day three and bronchopulmonary dysplasia (BPD) or death and further to assess the impact of patent ductus arteriosus (PDA) on this association in neonates born before 32 gestational weeks.

Methods
A cohort study of 183 neonates born before 32 gestational weeks consecutively admitted to the Neonatal Intensive Care Unit, Aarhus University Hospital, Denmark. On day three plasma samples were collected and echocardiography carried out. NT-proBNP was measured by routine immunoassays. The combined outcome BPD or death was assessed at 36 weeks of postmenstrual age. Receiver operator characteristic (ROC) analysis was performed to determine the discrimination ability of NT-proBNP by the natural log continuous measure to recognize BPD or death. The association of BPD or death was assessed in relation to natural log NT-proBNP levels day three.
Results
The risk of BPD or death increased 1.7-fold with one unit increase of natural log NT-proBNP day three when adjusted for gestational age at birth (OR = 1.7, 95% CI 1.3; 2.3). The association was found both in neonates with and without a PDA. Adjusting for GA, PDA diameter, LA:Ao-ratio, or early onset sepsis did not change the estimate.

Conclusion
We found NT-proBNP to be associated with BPD or death in very preterm neonates. This association was not only explained by the PDA. We speculate that NT-proBNP may help the identification of neonates at risk of BPD as early as postnatal day three.

Introduction
Bronchopulmonary dysplasia (BPD) is the most prevalent and one of the most serious complications of preterm birth with long-term consequences including pulmonary and neurodevelopmental impairment and post-neonatal mortality [1]. The pathogenesis of BPD is multifactorial and involves inflammation, lung injury, oxygen toxicity, and genetic predisposition although the relative contribution of each and their interplay have not been fully clarified [2,3]. Great interest lies in the potential to describe biomarkers that can identify neonates with the highest risk of BPD, enabling preventative strategies and trials of early treatment to modify risk.

B-type natriuretic peptide (BNP) and the inactive by-product N-terminal pro-B type natriuretic peptide (NT-proBNP) are cleaved from proBNP. The production and section is mediated by complex integration of mechanical, chemical, neuro-humeral, and immunological inputs [4,5]. BNP have diuretic, natriuretic, and vasodilatory properties and interacts with the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system [6–9]. NT-proBNP is more stable in plasma samples and has a longer half-life in circulation why it is the preferred biomarker compared to BNP. Plasma NT-proBNP level is associated with echocardiographic markers of a significant PDA [10,11]. However, there is little information about the pathophysiological role of NT-proBNP and BNP in neonatal disease [12–15].

Previous data suggests an association between plasma NT-proBNP, when measured at four weeks of age, and risk of BPD in preterm neonate [13]. This possible association may relate to inflammation, volume overload or high pulmonary arterial pressure. However, as the presence of a clinically significant PDA is associated with BPD [16–18] it is possible that the PDA, at least in part, may explain the association between NT-proBNP and BPD. A high volume shunt across a PDA will lead to increased pulmonary blood flow that may cause edema, increase pulmonary pressure and induce structural changes that could lead to BPD whereas the increased pulmonary venous return of blood to the heart may cause an increase in plasma NT-proBNP levels.

Accordingly, NT-proBNP may provide additional information in the evaluation of risk of morbidity in very preterm neonates in the immediate postnatal period. However, the interpretation and application need further exploration. The aim of this study was to evaluate the association between plasma NT-proBNP postnatal day three and BPD or death and further to assess if a possible association is explained by the PDA in very preterm newborns.
Methods

Study population
The study population has previously been described [18]. From June 1, 2010 to February 28, 2012 all newborns with a gestational age less than 32 completed weeks at birth admitted to our level three neonatal intensive care unit (NICU) were eligible for inclusion into this study. We excluded neonates with chromosomal abnormalities or congenital heart malformations other than atrium septum defects from the study.

Data collection and definitions
All neonates were assessed by echocardiography as part of their routine care. Blood samples were collected together with routine blood samples, to avoid extra needle sticks and excessive blood sampling. Blood sampling and echocardiography was performed on day three (median day three; IQR 3–4). Samples were stored as plasma at −80°C until analyses.

The following clinical information were obtained from the patients’ medical records; antenatal steroid administration, maternal preeclampsia, mode of delivery, multiple birth, weight and gestational age at birth (based on ultrasound), Apgar score at 1 and 5 minutes, surfactant administration, early onset sepsis (seven days of antibiotics initiated within the first three days after birth) [19,20], use of inotropes (within the first three days), and packed red blood cell transfusion (within the first three days), last day with oxygen supplementation, mechanical ventilation, and nasal continuous positive airway pressure (nCPAP). The primary outcome was BPD or death. BPD was defined as the need for oxygen supplementation or respiratory support at 36 weeks of postmenstrual age [17].

NT-proBNP measurements
NT-proBNP was measured in batch by routine immune-assay analysis on the Cobas e601 platform (Roche Diagnostics, Basel, Switzerland) at the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark. Electrochemiluminescent sandwich enzyme linked immunoabsorbant assays were used. The Elecsys pro-BNP II quantitative assay has a lower and upper detection limit of 5 and 35,000 ng/l, respectively (70,000 ng/l if diluted with Elecsys Diluent Universal) [21]. The assay complies with the DANAK ISO 15189 accreditation for Clinical Biochemical Laboratories.

Echocardiography measurements
Echo was performed by two senior pediatric echocardiographers (J Bjerre and MR Schmidt) using a Philips IE33 Ultrasound machine with a 12-MHz cardiology probe (Philips Healthcare, Andover, Massachusetts, USA. A complete echocardiography study was performed to confirm normal anatomy of the heart. Using standard neonatal windows [22] a PDA was defined as present if flow could be visualized by color Doppler. PDA diameter was measured in B mode at the most narrow point. A clinically significant PDA was defined as a PDA with a diameter of 1.5 mm or more and a small PDA as a PDA with a diameter below 1.5 mm [23,24]. The ratio of the left atrium to the aorta (LA:Ao-ratio) was determined in the parasternal long axis with M-mode using leading edge to leading edge method.

Clinical guidelines
The management of a PDA was according to a standardized departmental guideline. In accordance with these none of the neonates received Ibuprofen, Indomethacin or had surgical ligation performed before day three.
Statistical analysis

The distribution of NT-proBNP was skewed, and therefore natural log transformation was applied [25,26]. Data were presented as geometric mean and 95% confidence intervals (CI). Perinatal characteristics were described by number (percentage) or median values (interquartiles or range). The cohort was divided into quartiles of NT-proBNP to evaluate the frequency of BPD or death. Receiver operator characteristic (ROC) analysis was performed to determine the discrimination ability of NT-proBNP by the natural log continuous measure to recognize BPD or death. Odds ratios (OR) and 95% CI for BPD or death were assessed in relation to natural log NT-proBNP levels. Estimates were adjusted for co-variates chosen a priori; gestational age in weeks (continuous), PDA diameter (continuous), LA:Ao-ratio (continuous), early onset sepsis (dichotomized), mechanical ventilation (dichotomized), and presence of PDA (dichotomized). Robust cluster standard errors were used in order to take into account the correlation between twins. STATA special edition version 11 (College Station, Texas, USA) was used for analyses. All tests were two-sided. P-values of less than 0.05 were considered statistically significant.

Ethics

All parents gave informed, written consent for their child to participate in the study. The study was approved by the Central Denmark Region Committee on Health Research Ethics (journal number M-20090243), the Danish Data Protection Agency, and the National Board of Health.

Results

A total of 184 neonates born with a gestational age less than 32 weeks were admitted to the NICU during the study period. We consecutively obtained data on echocardiography and plasma NT-proBNP levels in 134 neonates (Fig 1). Of the 125 neonates who survived to a postmenstrual age of 36 weeks, 39 neonates (31%) were diagnosed with BPD. Neonates that were diagnosed with BPD or died had more unfavorable baseline characteristics compared to neonates with no BPD (Table 1).

We found that NT-proBNP levels day three were higher in neonates who developed BPD or died compared to neonates that did not (11,607 ng/l (95% CI: 8,053; 16,728) vs 3,495 ng/l (95% CI: 2,712; 4,504), p < 0.001). This finding was consistent in both neonates with a diagnosis of PDA on day three (18,901 ng/l (95% CI: 12,351; 28,924) vs 6,755 ng/l (95% CI: 4,039; 11,298), p < 0.001) and in neonates without PDA (4,770 ng/l (95% CI: 2,986; 7,621) vs 2,500 ng/l (95% CI: 1,951; 3,202), p < 0.05).

There was an increasing frequency of BPD or death from lowest to highest NT-proBNP quartiles (Table 2). Also, the frequency of PDA and especially a clinically significant PDA increased over NT-proBNP quartiles (Table 2). Multivariate logistic regression analysis showed that the odds of BPD or death adjusted for gestational age increased 1.7-fold (OR = 1.7, 95% CI: 1.3; 2.3) for every one unit increase in the natural logarithm of the concentration of NT-proBNP (one unit increase in the natural logarithm is equivalent to a 2.7 multiplication of the concentration on the ordinary scale). Excluding neonates born before 26 gestational weeks (n = 23) or neonates on mechanical ventilation

Fig 2 shows the receiver operating characteristic analysis distribution of plasma NT-proBNP in predicting BPD or death. The area under the curve for NT-proBNP was 0.76 (95% CI: 0.68; 0.84). The area under the curve for neonates with a clinically significant PDA was larger than the area for neonates with no PDA or a clinically insignificant PDA based on diameter (AUC day three no PDA = 0.70 (95% CI: 0.57–0.83) vs AUC clinically insignificant PDA = 0.64 (95% CI: 0.41–0.86) vs AUC clinically significant PDA = 0.82 (95% CI: 0.67–0.96).

Multivariate logistic regression analysis showed that the odds of BPD or death adjusted for gestational age increased 1.7-fold (OR = 1.7, 95% CI: 1.3; 2.3) for every one unit increase in the natural logarithm of the concentration of NT-proBNP (one unit increase in the natural logarithm is equivalent to a 2.7 multiplication of the concentration on the ordinary scale). Excluding neonates born before 26 gestational weeks (n = 23) or neonates on mechanical ventilation
day three (n = 20) did not change this estimate. In neonates with no PDA the odds of BPD or death was almost doubled for every one unit increase in the natural logarithm of the concentration of NT-proBNP when adjusted for GA (OR = 1.9; 95% CI: 1.1; 3.2) and increased by 4-fold in neonates with a clinically significant PDA (OR = 4.4; 95% CI: 1.3; 16), whereas no statistically significant association was found in neonates with a non-clinically significant PDA (OR = 1.2; 95% CI: 0.6; 2.4). Further adjusting for PDA diameter, LA:Ao-ratio, mechanical ventilation, and early onset sepsis did not influence the estimated association.

Discussion

We found that the risk of BPD or death was associated with plasma NT-proBNP as early as postnatal day three, also after adjusting for gestational age. We found that the association between BPD or death and NT-proBNP was strongest in neonates with a clinically significant PDA on day three. Further adjusting for LA:Ao-ratio, PDA diameter and early onset sepsis did not change this association. The association was also present in neonates with no PDA, suggesting that the association between BPD or death and NT-proBNP is not only explained by the PDA.

This is, to the best of our knowledge, the first study to investigate the relation between plasma NT-proBNP postnatal day three and BPD or death. Joseph et al. reported that higher...
plasma NT-proBNP measured in preterm neonates at four weeks of age was associated with an increased risk of BPD [13]. This was a pilot study including only 34 neonates, born with a gestational age less than 34 weeks. Neonates requiring supplemental oxygen at four weeks postnatal age were defined as having BPD. We defined BPD in accordance with most literature as a need for supplemental oxygen or respiratory support at 36 postmenstrual weeks [12,17]. In a study including a total of 136 neonates with a birth weight below 1500 g Czernik et al. found an association between urine NT-proBNP at the age of 7 days and BPD [12]. Recently evidence, from a cohort of 60 neonates born before 32 gestational weeks, concluded that BNP was associated with BPD at the time of diagnosis [27].

Increasing evidence supports the use of NT-proBNP levels as biomarkers in screening, diagnosis, management, and follow-up of children with cardiac disease[28]. NT-proBNP levels have been found to correlated with the magnitude of a left-to-right shunt[29]. Czernik et al. also described an association between NT-proBNP and retinopathy of the preterm (ROP) [12]. El-Khuffash et al. found that high NT-proBNP and troponin T levels in neonates with a PDA were associated with an increased risk of intraventricular hemorrhage (IVH) or death [14]. They further described that combining NT-proBNP and troponin T with echocardiographic measures of a significant PDA may facilitate the identification of neonates at risk of death or poor neurodevelopmental outcome at two years of age [15].

We decided, a priori, to explore the relation between PDA, NT-proBNP and BPD further in an attempt to understand this association. NT-proBNP levels are known to increase with presence of a PDA, increased PDA diameter and LA:Ao-ratio [30,31]. Pulmonary blood flow is

| Characteristics                        | no BPD n = 86 | BPD or death n = 48 | p-value  |
|----------------------------------------|---------------|---------------------|----------|
| GA weeks, median (range)               | 29 (24–31)    | 26 (23–31)          | < 0.01   |
| Birth weight g, median (range)         | 1170 (610–2090)| 835 (570–1672)      | < 0.01   |
| SGA, n (%)                             | 21 (24)       | 14 (29)             | ns       |
| Males, n (%)                           | 49 (57)       | 31 (65)             | ns       |
| Singleton, n (%)                       | 54 (63)       | 34 (71)             | ns       |
| Apgar at 1 min, median (IQR)           | 8 (5–9)       | 7 (5–8)             | ns       |
| Apgar at 5 min, median (IQR)           | 10 (9–10)     | 10 (8–10)           | < 0.05   |
| Surfactant, n (%)                      | 31 (36)       | 30 (62)             | < 0.01   |
| Sepsis, n (%)                          | 9 (10)        | 14 (29)             | < 0.01   |
| PRBC transfusion, n (%)                | 5 (6)         | 17 (35)             | < 0.01   |
| Inotropes, n (%)                       | 5 (6)         | 7 (15)              | ns       |
| Mechanical ventilation, n (%)          | 4 (4)         | 16 (33)             | < 0.01   |
| PDA, n (%)                             | 29 (34)       | 31 (65)             | < 0.01   |
| Clinically significant PDA, n (%)      | 14 (16)       | 20 (42)             | < 0.01   |
| Preeclampsia, n (%)                    | 19 (22)       | 10 (21)             | ns       |
| Antenatal steroids, n (%)              | 82 (96)       | 33 (91)             | ns       |
| Cesarean delivery, n (%)               | 56 (65)       | 31 (65)             | ns       |

GA gestational age, SGA small for gestational age defined as birth weight below -2SD according to the formula by Marsal er al., Sepsis defined as seven days of antibiotics initiated before day three, PRBC paced red blood cell transfusion within first three days of life, Inotropes used within the first three days of life, Mechanical ventilation day three, PDA patent ductus arteriosus day three, Clinically significant PDA if diameter ≥1.5 mm

doi:10.1371/journal.pone.0140079.t001

Table 1. Characteristics of 134 neonates born before 32 gestational weeks by presence of bronchopulmonary dysplasia (BPD) or death at 36 weeks of postmenstrual age.
increased by an aortic-pulmonary shunt across a PDA, therein increasing venous return to the heart and this may increase left atrium and ventricular pressure and size [23,32]. This is known to increase synthesis and release of BNP and hence NT-proBNP [7]. Neither of the studies by Joseph et al. or Czernik et al. provided information on attributes of the PDA prior to 4 weeks of postnatal age. We found that the association between NT-proBNP and BPD or death was present regardless of the presence of a PDA on day three. In addition the estimate of the association did not change when corrected for PDA diameter or LA:Ao-ratio. However, the strongest association between NT-proBNP and BPD or death was found in neonates with a clinically significant PDA day three. This suggests that the presence of a PDA does not solely explain the increased NT-proBNP levels, but re-affirms the need to consider other aspects of hemodynamic significance as the presence of a clinically significant PDA does modify the association between NT-proBNP and BPD or death. These data align with recent reports that suggest that PDA shunt volume may be a more clinically relevant end-point of interest, rather than

### Table 2. Plasma NT-proBNP quartiles postnatal day three in relation to PDA status and morbidity in neonates born before 32 gestational weeks, Aarhus University Hospital, Denmark.

|           | Q1           | Q2           | Q3           | Q4           |
|-----------|--------------|--------------|--------------|--------------|
| Quartile range (ng/l) | 500-1,927 | 1,955 - 3,789 | 3,906- 15,977 | 16,414- 70,000 |
| GA weeks, median (IQR) | 30 (29–31) | 28 (27–30) | 28 (26–29) | 27 (25–28) |
| PDA, n (%) | 7 (21) | 7 (21) | 16 (48) | 30 (88) |
| Clinically significant PDA, n (%) | 0 (0) | 5 (15) | 6 (18) | 23 (68) |
| Sepsis, n (%) | 2 (6) | 2 (6) | 10 (30) | 9 (26) |
| Mechanical ventilation, n (%) | 0 (0) | 3 (9) | 6 (18) | 11 (32) |
| Inotropes, n (%) | 1 (3) | 3 (9) | 2 (6) | 6 (18) |
| BPD or death, n (%) | 3 (9) | 9 (26) | 13 (38) | 23 (67) |

NT-proBNP N-Terminal pro-Brain Natriuretic peptide maximum detection limit of 70,000 ng/l. PDA patent ductus arteriosus day three. GA gestational age. Clinically significant PDA if diameter ≥1.5 mm. Sepsis defined as seven days of antibiotics initiated before day three. Inotropes used within the first three days of life. Mechanical ventilation postnatal day three. BPD bronchopulmonary dysplasia.

doi:10.1371/journal.pone.0140079.t002

Fig 2. Receiver operator characteristics curve of plasma NT-proBNP day three in 134 neonates born before 32 gestational weeks to predict BPD at 36 weeks of postmenstrual age or death. Area under the curve (AUC) for NT-proBNP in predicting BPD or death (AUC = 0.76, 95% CI: 0.68; 0.84).

doi:10.1371/journal.pone.0140079.g002
diameter alone which is subject to operator dependent error and does not consider transductal resistance patterns. The lack of impact of the LA:Ao-ratio on the association, may relate to the confounding effect of a large left-to-right transatrial shunt which is commonly associated with increased PDA shunt volume [33].

Presence of a PDA and especially a PDA with a diameter larger than 1.5 mm on day three is known to be associated with BPD [16,18]. Excessive pulmonary blood flow and pressure in an immature lung with ongoing maturation of alveolar and vascular structures may result in abnormal development of the pulmonary vessels [34]. However, a causal relationship between PDA and BPD has not been proven [18]. Infection [35] and pulmonary inflammation may play a crucial role in the development of BPD [36]. In vitro studies have suggested that cytokines are involved in NT-proBNP release [37,38]. In adults plasma BNP levels have been found to be associated with severe sepsis [39]. We found that adjusting for early onset sepsis did not change the described association between NT-proBNP and BPD or death.

The heart exerts an endocrine function and has a role in the regulation of both cardiovascular and renal systems [4,40]. BNP has been found to increase after birth and reach a plateau at day three to four [41]. The presence of a high volume PDA shunt or sustained elevation in pulmonary blood pressure [42] as part of physiological transition from fetal to postnatal circulation may cause this increase [41]. Water and sodium handling has been found to be different at four weeks of age in neonates that develop BPD compared to neonates without BPD [43]. In very low birth weight infants higher water intake and less weight loss during the first ten days of life have been found to be associated with an increased risk of BPD [44,45]. The release of pro-BNP and hence BNP and NT-proBNP may reflect a response to volume overload and an augmentation of the RAAS system. Further investigations into these complex integrative regulatory mechanisms would be of great interest in order not only to evaluate the use of NT-proBNP as a biomarker but also to describe the pathogenesis of BPD.

Strengths and limitations

We consecutively included neonates admitted to the NICU. Echocardiography and plasma samples were collected systematically very early in life and prior to the outcome of interest. BPD or death was used as the outcome rather than BPD because death is a competing outcome with BPD [46]. BPD was defined as need for supplemental oxygen or respiratory support by 36 weeks of postmenstrual age. Often ventilator support is used in the definition of BPD, but as none of the neonates needed mechanical ventilation at this point we reported also the need for supplemental oxygen and nCPAP.

Neonates that were diagnosed with BPD or died were born at a lower gestational age compared to neonates with no BPD. To make groups more comparable we made sub-analysis excluding neonates born before 26 gestational weeks and found that the association between BPD or death and plasma NT-proBNP was unchanged.

In 26 neonates that had an echocardiography we did not obtain plasma samples. This was in some cases due to the order for the blood samples not being communicated but may possibly also have been related to neonates that were not stable enough for blood sampling and could thus affect generalizability. In five neonates plasma NT-proBNP was above the detection limit of 70,000 ng/l they were in the analysis set to the value of 70,000 ng/l. All of these neonates died or had BPD by 36 weeks of postmenstrual age.

Conclusion

We found increased NT-proBNP levels as early as day three of life to be associated with an increased risk of BPD or death. The strongest association was found in neonates with a
clinically significant PDA; however, it was also present in neonates with no PDA. The association remained unchanged after adjusting for PDA diameter, LA:Ao-ratio and perinatal characteristics suggesting that NT-proBNP may be helpful in PDA treatment selection and as an early marker of BPD in very preterm neonates.

Acknowledgments
The authors would like to thank the parents of the infants enrolled, the staff at the neonatal intensive care unit at Aarhus University Hospital, Aarhus, and a special thanks to laboratory technician Jane Knudsen for handling plasma samples.

Author Contributions
Conceived and designed the experiments: AS TBH VEH BHB. Analyzed the data: AS TBH VEH BHB PJM. Contributed reagents/materials/analysis tools: JVB MRS TBH BHB. Wrote the paper: AS TBH VEH BHB PJM JVB MRS. Performed echocardiography: JVB MRS.

References
1. Doyle LW, Anderson PJ. Long-term outcomes of bronchopulmonary dysplasia. Semin Fetal Neonatal Med 2009; 14: 391–395. doi: 10.1016/j.siny.2009.08.004 PMID: 19766550
2. Korhonen P, Tammela O, Koivistio AM, Laippala P, Ikonen S. Frequency and risk factors in bronchopulmonary dysplasia in a cohort of very low birth weight infants. Early Hum Dev 1999; 54: 245–258. PMID: 10321791
3. Bancalari E, Claire N, Sosenko IR. Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology and definition. Semin Neonatal 2003; 8: 63–71. PMID: 12667831
4. Clerico A, Giannoni A, Vittorini S, Passino C. Thirty years of the heart as an endocrine organ: physiological role and clinical utility of cardiac natriuretic hormones. Am J Physiol Heart Circ Physiol 2011; 301: H12–H20. doi: 10.1152/ajpheart.00226.2011 PMID: 21551272
5. Del RS, Cabiati M, Clerico A. Recent advances on natriuretic peptide system: new promising therapeutic targets for the treatment of heart failure. Pharmaco Res 2013; 76: 190–198. doi: 10.1016/j.phrs.2013.08.006 PMID: 23988785
6. Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994; 90: 195–203. PMID: 8025996
7. de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. Lancet 2003; 362: 316–322. PMID: 12892964
8. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med 1998; 339: 321–328. PMID: 9682046
9. Weber M, Hamm C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. Heart 2006; 92: 843–849. PMID: 16698841
10. Farombi-Oghuvbu I, Matthews T, Mayne PD, Guerin H, Corcoran JD. N-terminal pro-B-type natriuretic peptide: a measure of significant patent ductus arteriosus. Arch Dis Child Neonatal Ed 2008; 93 (4): F257–F260. doi: 10.1136/adc.2007.120691 PMID: 18218660
11. El-Khuffash AF, Molloy EJ. Influence of a patent ductus arteriosus on cardiac troponin T levels in preterm infants. J Pediatr 2008; 153(3): 350–353. doi: 10.1016/j.jpeds.2008.04.014 PMID: 18534211
12. Czernik C, Metze B, Muller C, Muller B, Buhrer C. Urinary N-terminal B-type natriuretic peptide predicts severe retinopathy of prematurity. Pediatrics 2011; 128: e545–e549. doi: 10.1542/peds.2011-0603 PMID: 21824875
13. Joseph L, Nir A, Hammeman C, Goldberg S, Ben SE, Picard E. N-terminal pro-B-type natriuretic peptide as a marker of bronchopulmonary dysplasia in premature infants. Am J Perinatol 2010; 27: 381–386. doi: 10.1055/s-0029-1243312 PMID: 20013606
14. El-Khuffash A, Barry D, Walsh K, Davis PG, Molloy EJ. Biochemical markers may identify preterm infants with a patent ductus arteriosus at high risk of death or severe intraventricular haemorrhage. Arch Dis Child Neonatal Ed 2008; 93: F407–F412. doi: 10.1136/adc.2007.133140 PMID: 18285375
15. El-Khuffash AF, Stevin M, McNamara PJ, Molloy EJ. Troponin T, N-terminal pro natriuretic peptide and a patent ductus arteriosus scoring system predict death before discharge or neurodevelopmental outcome at 2 years in preterm infants. Arch Dis Child Fetal Neonatal Ed 2011; 96(2): F133–F137. doi: 10.1136/adc.2010.185967 PMID: 21071684

16. Landry JS, Menzies D. Occurrence and severity of bronchopulmonary dysplasia and respiratory distress syndrome after a preterm birth. Paediatr Child Health 2011; 16: 399–403. PMID: 22851893

17. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001; 163: 1723–1729. PMID: 11401896

18. Sellmer A, Bjerre JV, Schmidt MR, McNamara PJ, Hjortdal VE, Host B, et al. Morbidity and mortality in preterm neonates with patent ductus arteriosus on day 3. Arch Dis Child Fetal Neonatal Ed 2013; 98: F505–F510. doi: 10.1136/archdischild-2013-303816 PMID: 23893268

19. Stoll BJ, Gordon T, Korones SB, Shankaran S, Tyson JE, Bauer CR, et al. Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr 1999; 129: 72–80. PMID: 8757565

20. Simonson KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clin Microbiol Rev 2014; 27: 21–47. doi: 10.1128/CMR.00031-13 PMID: 24396135

21. Prontera C, Zucchelli GC, Vittorini S, Storti S, Emdin M, Clerico A. Comparison between analytical performances of polyclonal and monoclonal electrochemiluminescence immunoassays for NT-proBNP. Clin Chim Acta 2009; 400: 70–73. doi: 10.1016/j.cca.2008.10.011 PMID: 18992732

22. Lai WW, Geva T, Shirali GS, Frommelt PC, Humes RA, Brook MM, et al. Guidelines and standards for performance of a pediatric echocardiogram: a report from the Task Force of the Pediatric Council of the American Society of Echocardiography. J Am Soc Echocardiogr 2006; 19: 1413–1430. PMID: 17138024

23. El Hajjar M, Vaksmann G, Rakza T, Kongolo G, Storme L. Severity of the ductal shunt: a comparison of different markers. Arch Dis Child Fetal Neonatal Ed 2005; 90: F419–F422. PMID: 16113155

24. Sehgal A, McNamara PJ. Does echocardiography facilitate determination of hemodynamic significance attributable to the ductus arteriosus? Eur J Pediatr 2009; 168(8): 907–914. doi: 10.1007/s00431-009-0983-3 PMID: 19387684

25. Nir A, Lindinger A, Rauh M, Bar-Oz B, Laer S, Schwachtgen L, et al. NT-pro-B-type natriuretic peptide in infants and children: reference values based on combined data from four studies. Pediatr Cardiol 2009; 30(1): 3–8. doi: 10.1007/s00246-008-9258-4 PMID: 18600369

26. Klersy C, d'Eril GV, Barassi A, Palladini G, Comelli M, Moratti R, et al. Advantages of the lognormal approach to determining reference change values for N-terminal propeptide B-type natriuretic peptide. Clin Chim Acta 2012; 413: 544–547. doi: 10.1016/j.cca.2011.11.012 PMID: 22155398

27. Kalra VK, Aggarwal S, Arora P, Natarajan G. B-type natriuretic peptide levels in preterm neonates with bronchopulmonary dysplasia: A marker of severity? Pediatr Pulmonol 2013; 49(11): 1106–11. doi: 10.1002/ppul.22942 PMID: 24214578

28. Cantinotti M, Walters HL, Crocetti M, Marotta M, Murzi B, Clerico A. BNP in children with congenital cardiac disease: is there now sufficient evidence for its routine use? Cardiol Young 2015; 25: 424–437. doi: 10.1016/S1047-9511(14)00213-3 PMID: 24730107

29. Cantinotti M, Law Y, Vittorini S, Crocetti M, Marco M, Murzi B, et al. The potential and limitations of plasma BNP measurement in the diagnosis, prognosis, and management of children with heart failure due to congenital cardiac disease: an update. Heart Fail Rev 2014; 19: 727–742. doi: 10.1007/s10741-014-9422-2 PMID: 24473828

30. El-Khuffash AF, Amoroso M, Culliton M, Molloy EJ. N-terminal pro-B-type natriuretic peptide as a marker of ductal haemodynamic significance in preterm infants: a prospective observational study. Arch Dis Child Fetal Neonatal Ed 2007; 92: F421–F422.

31. Nuntunarumit P, Chongkongkiat P, Khositseth A. N-terminal-pro-brain natriuretic peptide: a guide for early targeted indomethacin therapy for patent ductus arteriosus in preterm Infants. Acta Paediatr 2011; 110: 1217–1221. doi: 10.1111/j.1651-2227.2011.02304.x PMID: 21457304

32. Iyer P, Evans N. Re-evaluation of the left atrial to aortic root ratio as a marker of patent ductus arteriosus. Arch Dis Child Fetal Neonatal Ed 1994; 70(2): F112–F117. PMID: 8154903

33. Evans N, Iyer P. Assessment of ductus arteriosus shunt in preterm infants supported by mechanical ventilation: effect of interatrial shunting. J Pediatr 1994; 125(5 Pt 1): 778–785. PMID: 7965434

34. Mata-Greenwood E, Meyrick B, Soifer SJ, Fineman JR, Black SM. Expression of VEGF and its receptors Flt-1 and Flk-1/KDR is altered in lambs with increased pulmonary blood flow and pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2003; 285: L222–L231. PMID: 12665467
35. Gonzalez A, Sosenko IR, Chandar J, Hummler H, Claure N, Bancalari E. Influence of infection on patient ductus arteriosus and chronic lung disease in premature infants weighing 1000 grams or less. J Pediatr 1996; 128: 470–478. PMID: 8618179

36. Merritt TA, Deming DD, Boynton BR. The ‘new’ bronchopulmonary dysplasia: challenges and commentary. Semin Fetal Neonatal Med 2009; 14: 345–357. doi: 10.1016/j.siny.2009.08.009 PMID: 19747889

37. Tanaka T, Kanda T, Takahashi T, Saegusa S, Moriya J, Kurabayashi M. Interleukin-6-induced reciprocal expression of SERCA and natriuretic peptides mRNA in cultured rat ventricular myocytes. J Int Med Res 2004; 32: 57–61. PMID: 14997707

38. de Bold AJ. Cardiac natriuretic peptides gene expression and secretion in inflammation. J Investig Med 2009; 57: 29–32. doi: 10.231/JIM.0b013e3181948b37 PMID: 19158604

39. Rudiger A, Gasser S, Fischer M, Homemann T, von EA, Maggiorini M. Comparable increase of B-type natriuretic peptide and amino-terminal pro-B-type natriuretic peptide levels in patients with severe sepsis, septic shock, and acute heart failure. Crit Care Med 2006; 34: 2140–2144. PMID: 16763507

40. Ogawa T, de Bold AJ. The heart as an endocrine organ. Endocr Connect 2014; 3: R31–R44. doi: 10.1530/EC-14-0012 PMID: 24562677

41. da Graca RL, Hassinger DC, Flynn PA, Sison CP, Nesin M, Auld PA. Longitudinal changes of brain-type natriuretic peptide in preterm neonates. Pediatrics 2006; 117(6): 2183–2189. PMID: 16740863

42. Reynolds EW, Ellington JG, Vranicar M, Bada HS. Brain-type natriuretic peptide in the diagnosis and management of persistent pulmonary hypertension of the newborn. Pediatrics 2004; 114: 1297–1304. PMID: 15520111

43. Kojima T, Fukuda Y, Hirata Y, Matsuzaki S, Kobayashi Y. Changes in vasopressin, atrial natriuretic factor, and water homeostasis in the early stage of bronchopulmonary dysplasia. Pediatr Res 1990; 27: 260–263. PMID: 2138727

44. Oh W, Poindexter BB, Perritt R, Lemons JA, Bauer CR, Ehrenkranz RA, et al. Association between fluid intake and weight loss during the first ten days of life and risk of bronchopulmonary dysplasia in extremely low birth weight infants. J Pediatr 2005; 147: 786–790. PMID: 16356432

45. Guo MM, Chung CH, Chen FS, Chen CC, Huang HC, Chung MY. Severe Bronchopulmonary Dysplasia is associated with Higher Fluid Intake in Very Low-Birth-Weight Infants: A Retrospective Study. Am J Perinatol 2015; 30(2):155–62. doi: 10.1055/s-0034-1376393 PMID: 24915556

46. Andersen PK, Geskus RB, de WT, Putter H. Competing risks in epidemiology: possibilities and pitfalls. Int J Epidemiol 2012; 41: 861–870. doi: 10.1093/ije/dyr213 PMID: 22253319