Published in final edited form as:

Congenit Heart Dis. 2012 May ; 7(3): 259–267. doi:10.1111/j.1747-0803.2011.00620.x.

B-type natriuretic peptide and amino-terminal pro-B-type natriuretic peptide in pediatric patients with pulmonary arterial hypertension

Shinichi Takatsuki, MD^{1,3}, Brandie D Wagner, PhD², and David Dunbar Ivy, MD¹

¹Department of Pediatrics, University of Colorado Denver School of Medicine, Children's Hospital Colorado

²Department of Biostatistics and Informatics, Colorado School of Public Health, University of Colorado Denver

Abstract

Objectives—B-type natriuretic peptide (BNP) and the amino-terminal fragment (NTproBNP) correlate with clinical variables, but have not been simultaneously studied in a large number of pediatric patients with pulmonary arterial hypertension (PAH). The purpose of our investigation was to compare BNP and NTproBNP with clinical indicators of disease in a pediatric PAH population for which biomarkers are much needed.

Design—We retrospectively compared BNP and NTproBNP levels with exercise capacity, echocardiographic data, and hemodynamics in PAH patients under 21 years-old. Two hundred sixty three blood samples from 88 pediatric PAH patients were obtained, with BNP and NTproBNP drawn at the same time.

Results—There was a correlation between BNP and NTproBNP with mean pulmonary arterial pressure/mean arterial pressure (mPAP/mSAP) ratio ($r=0.40$ $p<0.01$, $r=0.45$ $p<0.01$, respectively), mean right atrial pressure (mRAP) ($r=0.48$ $p<0.01$, $r=0.48$ $p<0.01$), and tricuspid regurgitant (TR) velocity ($r=0.36$ $p<0.01$, $r=0.41$ $p<0.01$). BNP and NTproBNP are associated with 6 minute walking distance, mPAP, mPAP/mSAP ratio, mRAP, pulmonary vascular resistance index (PVRI), and TR velocity when investigated longitudinally. On the average, a 1 unit increase in log BNP or NTproBNP was associated with 4.5 units $\times$ m² or 3.4 units $\times$ m² increase in PVRI, respectively. There was a strong correlation between log BNP and log NTproBNP measurements ($r=0.87$, $p<0.01$).

Conclusion—In pediatric PAH, BNP and NTProBNP are strongly correlated and predict changes in clinical variables and hemodynamics. In a cross-sectional analysis, NTproBNP correlated with echocardiographic and exercise data better than BNP; NTproBNP showed less within patient variability over time, therefore NTproBNP can add additional information towards predicting these clinical measurements.

Keywords

pulmonary arterial hypertension; B-type natriuretic peptide; amino-terminal fragment B-type natriuretic peptide; children

Corresponding Author; SHINICHI TAKATSUKI, Address 13123 East 16th Avenue, B100, Pediatric Cardiology, Children's Hospital Colorado and University of Colorado Denver school of Medicine, Aurora, CO 80045, tekeshin0621@msn.com Phone 7207774522, FAX 7207777290.

Disclosures: The authors have no conflicts of interest to disclose.

Introduction

Three major natriuretic peptides (NPs) have been discovered including atrial natriuretic peptide, B-type natriuretic peptide (BNP), and C-type natriuretic peptide. These NPs are activated to protect from volume or pressure overload in cardiovascular disease. The overload of the right or left ventricle leads to synthesis of the intracellular prohormone in the myocardium. The pro-BNP is cleaved into the biologically active hormone, BNP, and an inactive amino-terminal fragment (NTproBNP).^{1,2} Despite BNP and NTproBNP being equally secreted, their plasma concentrations are not 1:1. BNP has a half-life of approximately 20 min; in contrast, NTproBNP, has a longer half-life of approximately 1 to 2 h. The difference causes NTproBNP to have a higher circulating level and slower fluctuations compared with BNP.³ BNP has a potential role by counteracting increased ventricular volume due to vasoconstriction, sodium retention, and activation of the renin-angiotensin-aldosterone system.⁴ The measurement of BNP and NTproBNP are utilized in the decision making process in various clinical settings. The diagnostic value of both peptides has been well established in symptomatic and/or asymptomatic patients with chronic heart failure.^{5–10} BNP has important clinical implications to identify high-risk pulmonary arterial hypertension (PAH) adults and to guide treatment.¹¹ In addition, elevated plasma BNP concentrations are associated with increased mortality in patients with PAH.^{12–14} Likewise, NTproBNP can also be of prognostic value in predicting poor long-term outcome in patients with severe PAH.^{15–17} In addition, NTproBNP is cleared by the kidney and may be falsely evaluated with significant renal disease. Few studies have directly compared BNP and NTproBNP, and these comparisons have not been made in PAH. The purpose of our investigation was to compare BNP and NTproBNP with clinical indicators of disease in a pediatric PH population for which biomarkers are much needed.

Methods

This single-center observational study was performed between November, 2007 and October, 2010 at The Children's Hospital, Denver. All patients were enrolled in an IRB approved protocol entitled Prospective Evaluation of Adolescents and Children with Pulmonary Arterial Hypertension (PEACH). In total, there were 88 PAH patients with a median age of 10 years-old (IQR 5–15 years-old) with 46 (52%) of patients being female. Only observations with both BNP and NTproBNP measured at the same time were included. Observations from patients older than 21 years of age were excluded. All patients had a mean pulmonary artery pressure (mPAP) over 25 mmHg, pulmonary capillary wedge pressure under 15 mmHg at right heart catheterization, normal systemic blood pressure, and renal function. PAH due to ischemic heart diseases, cardiomyopathy, and pulmonary fibrosis were excluded by further diagnostic tests including echocardiography, high-resolution computed tomography scan, and pulmonary function tests.

The different etiologies of PAH were as follows: idiopathic PAH (n=35), PAH associated with congenital heart disease (n=39), and PAH associated with connective tissue disease (n=1). Two-dimensional echocardiographic data included tricuspid regurgitant (TR) jet velocities for estimating pulmonary artery systolic pressure, right ventricular diastolic dimension, and left ventricular fractional shortening (LVFS) by the modified Simpson's method. Right heart catheterization was performed with a balloon tipped, flow directed Swan-Ganz catheter. We measured mean right atrial pressure (mRAP), mPAP, mean systemic arterial pressure (mSAP), and pulmonary capillary wedge pressure. The pulmonary vascular resistance index (PVRI) was calculated. Cardiac output was obtained using thermodilution and cardiac index (CI) was calculated. If the patient had a significant intra-cardiac defect, cardiac output was obtained by the Fick method with oxygen consumption assessed by the LaFarge method. Pulmonary function tests were performed under room air at rest. We

measured peak oxygen consumption (peak VO₂) during cardiopulmonary test and calculated a ratio of minute ventilation (VE) to carbon dioxide output (VCO₂) as ventilator efficiency (VE/VCO₂).

The plasma BNP and serum NTproBNP level were obtained within 5 days of right heart catheterization. Echocardiographic evaluation, six-minute walk test with the maximum distance (6MWD), and cardiopulmonary exercise test within 90 days around the blood draw were included. In general, for those patients undergoing cardiac catheterization, the blood test was performed during the procedure. Echocardiography, pulmonary function tests, and 6MWD were most often performed the day before the catheterization. Blood for measurement of plasma BNP concentrations was collected in ethylenediaminetetraacetic acid tubes. In contrast, NTproBNP was collected in a serum sample tube. Plasma BNP was assayed on i-STAT® system using the two-site enzyme-linked immunosorbant assay (Abbott Laboratories, IL, USA) and serum NTproBNP level was measured on an electrochemiluminescence immunoassay (Mayo medical laboratories; ProBNP II, Roche Diagnostics, Indianapolis, IN, USA). Temporal changes were expressed as percentage of the baseline values. The minimum measurable level of BNP is 15 pg/ml.

Statistical Analyses

The marker measurements were log (base 10) transformed and anchored at 1. Descriptive statistics were calculated using mean $\pm$ standard deviations or medians and inter-quartile range (IQR) for continuous variables and percentages for categorical variables. Cross-sectional correlations were determined using *Spearman's rank correlation coefficients*. Associations were quantified using slope estimates from mixed effect regression models with a random subject effect to address the serial measurements contained in the full dataset. To estimate and compare the gender specific changes with age in the BNP and NTproBNP marker variables simultaneously, a bivariate mixed model with piece-wise linear regression and subject specific coefficients was used.¹⁸ All analyses were performed using SAS version 9.2 software (SAS Institute Inc., Cary, NC, 2008). A p-value of < 0.01 was considered to be statistically significant.

Results

Patient Demographics and clinical measurements

Clinical characteristics of the 88 pediatric patients using data from their first observation are shown in Table 1. Fifty-two % of patients were female between the ages of 1 month and 21 years. During the median follow-up period of 13.3 months (range 0.2 to 34 months), only 3 patients (3%) were initiated on epoprostenol infusion therapy. All patients survived owing to various vasodilator therapies; in addition, none of the patients received lung transplantation. The median mPAP was 38 mmHg (interquartile range (IQR), 27 to 54 mm Hg), the median mRAP value of 6.0 mm Hg (IQR, 4 to 8 mm Hg), the median PVRI was 7.5 units xm² (IQR; 4.2–12.3 units xm²), and the median CI was 4.1 l/min/m² (IQR; 3.3–5.3 l/min/m²). In our series, we obtained 263 blood samples from 88 patients. The median plasma BNP concentration was 38 pg/ml (IQR; 20–75 pg/ml) and median serum NTproBNP concentration was 179 pg/ml (IQR; 72–393 pg/ml).

The gender specific change in marker concentrations versus age was investigated by visual inspection of the plotted data with a scatterplot smoother to describe the general trend (Figure 1; 263 samples in 88 patients). Until around age 12 there is a decreasing trend in both NPs markers with increasing age without large gender differences. After the age of 12, the NPs concentrations for males begin to increase whereas the concentrations in the females decreased more slowly. A bivariate mixed effects model was used to estimate and compare

these trends across gender and marker (BNP versus NTproBNP). A piece-wise linear regression with a knot at 12 years old was used to describe the trends versus age. NTproBNP had significantly higher concentrations compared to BNP across the full age range. The slope of the BNP and NTproBNP levels decreased up to age 12, but was not significantly different between BNP and NTproBNP ($p=0.15$). After age 12, both NP concentrations begin to increase in males. This increase was faster than the increase in females, although these gender differences were not statistically significant (BNP $p = 0.56$, NTproBNP $p = 0.80$).

Cross-sectional correlations

This analysis utilizes the most recent sample pair from each patient, allowing each patient to equally contribute to the correlation estimate. There was a strong correlation between log BNP and log NTproBNP measurements with a cross-sectional correlation coefficient equal to 0.87 ($p<0.01$). Although the measurements are highly correlated, the NTproBNP values are detected in higher quantities compared to BNP, as expected (Figure 2; 88 samples in 88 patients).

Table 2 shows the correlations between both peptide levels and clinical variables using cross-sectional analysis. The NTproBNP levels had a higher correlation with 6MWD compared to BNP levels ($r = -0.49$, $p<0.01$, $r = -0.32$, $p=0.04$, respectively). Both NTproBNP and BNP were significantly associated with mPAP/mSAP ($r = 0.45$, $p<0.01$, $r = 0.40$, $p=0.01$, respectively), mRAP ($r=0.48$, $p<0.01$, $r=0.48$, $p<0.01$, respectively) and TR velocity ($r=0.41$, $p<0.01$, $r=0.36$, $p<0.01$, respectively). Interestingly, hemodynamic parameters, including mPAP and PVRI, were not significantly associated with NPs in PAH patients. With sensitivity analysis, the correlations were calculated using only measurements taken on the same day as blood collection. The correlations between 6MWD and the markers became more comparable (BNP $r = -0.41$ and NTproBNP $r = -0.42$), the difference between correlations with LVFS doubled (BNP $r = 0.54$ and NTproBNP $r = 0.26$) while the correlations with other echo markers were not as dramatically affected. For all the hemodynamic variables, except CI, BNP continued to have higher correlations compared to NTproBNP (mPAP; $r = 0.37$, $p=0.02$, $r = 0.26$, $p=0.11$, PVRI; $r = 0.23$, $p=0.16$, $r = 0.13$, $p=0.45$, respectively). There were not adequate samples sizes to perform the sensitivity analysis for the exercise variables.

Associations between serial natriuretic peptide measurements and clinical variables

Using all patient data available, assessment of conditional associations was performed to determine the association between the clinical variables with a 1 unit change in log BNP or NTproBNP (Figure 3). The t-statistic corresponding to the slope estimate was used to compare the magnitude of the associations between BNP and NTproBNP. The sign of the t-values correspond to the direction of the association. For example, the negative 6MWD t-value indicates that as log BNP increases the 6MWD decreases. NTproBNP had larger t-values for 6MWD, TR velocity, peak VO₂ and VE/VCO₂ compared to BNP. Whereas BNP had only slightly larger t-values for mPAP and CI compared to NTproBNP.

Table 3 shows the associations between BNP markers and clinical variables using all patient data accounting for repeated measures. Both peptides, BNP and NTproBNP were associated with 6MWD (BNP; slope = -49.0 m, 99% confidence limits (CL); -88.1 - -9.9, NTproBNP; -49.7 m, CL -84.0 - -15.4), mPAP (BNP; 12.9 mmHg, CL 3.1-22.6, NTproBNP; 9.0 mmHg, CL 1.23-16.9), mPAP/mSAP (BNP; 0.24, CL 0.08-0.40, NTproBNP; 0.20, CL 0.07-0.32), mRAP (BNP; 3.0 mmHg, CL 1.3-4.7, NTproBNP; 2.5 mmHg, CL 1.2-3.8), PVRI (BNP; 4.5 unitsxm², CL 0.4-8.7, NTproBNP; 3.4 unitsxm², CL 0.1-6.6), and TR velocity (BNP; 0.34m/s, CL 0.07-0.60, NTproBNP; 0.40m/s CL 0.17-

0.62) (Figure 4; 214 samples in 69 patients). In this study, there was less variability in the association estimates of NTproBNP compared to BNP, which can be seen by comparing the standard error of estimates. Intraclass correlation coefficients (ICC) were calculated for each marker, BNP and NTproBNP had ICC values of 0.52 and 0.61, respectively. The ICC corresponds to the proportion of total variation that is between subjects, with a higher value indicating the concentrations within a patient are more similar. The associations were re-calculated after adjusting for the potential confounding effects of age and gender due to the results presented earlier related to the significant changes in the markers with the variables. The magnitude and significance of all associations were robust after adjusting for age and gender.

Discussion

In this single center retrospective cohort study, we sought to investigate and compare the characteristics between BNP and NTproBNP in pediatric PH patients with variations in disease severity. We found that BNP and NTproBNP levels had a strong correlation with each other and there were significant age and gender related differences in both peptides. Additionally, both peptides significantly, but moderately correlated with TR velocity and hemodynamics such as mRAP, when considered in a cross sectional analysis. NTproBNP correlated better with variables not collected on the same day, such as 6MWD and echocardiographic data, moreover, the difference in the correlation estimates lessened with the constraint that the measurements be taken on the same day. In contrast, BNP tended to correlate better with the catheterization variables such as mPAP and PVRI as the blood samples were collected during catheterization. Therefore, NTproBNP may have advantages if the time between collection and measurements are greater owing to a longer half-life. However, mPAP and PVRI were significantly associated with both peptides when evaluated longitudinally. The longitudinal associations yielded similar results. None of the associations with the clinical variables were statistically different between BNP and NTproBNP. Our studies suggested both markers may be equally useful for making decisions in a clinical setting. This study is interesting because this is the first report to evaluate both BNP and NTproBNP in a relatively large pediatric PAH population.

Previous studies in PAH have shown that each peptide significantly correlated with hemodynamics. A circulating BNP was significantly correlated with mPAP ($r = 0.73$), total pulmonary resistance ($r = 0.79$), and mean RAP ($r = 0.79$) in 26 patients with idiopathic or thromboembolic pulmonary hypertension,¹⁹ but the number of patients in this study was small and the population was more clearly defined compared the population used here. In addition, elevated BNP level was a significant prognostic factor.^{13,20} Likewise, the elevation of serum NTproBNP level was useful in identifying the poor long-term prognosis in idiopathic PAH.^{13,16,17}

Both peptides can be easily and non-invasively measured, therefore, they are widely used as reliable biomarkers in patients with PAH. However, there are few data comparing BNP and NTproBNP. The elimination of BNP in plasma is shorter compared with NTproBNP.²¹ For this reason, the plasma BNP level may be more useful for assessing more real-time hemodynamic changes in pediatric patients with PAH. In contrast, NTproBNP is eliminated only via the kidneys, resulting in a significantly longer half-life^{22,23} and higher concentrations compared to BNP. A previous report showed that such different clearance mechanisms might contribute to the superiority of NTproBNP as a mortality marker.²⁴ In our present study, we were unable to determine whether BNP and NTproBNP is a more sensitive predictor of outcome. However, our results performed on the concentrations collected serially over time, indicate the increased stability of NTproBNP concentrations compared to BNP and would corroborate this finding.

BNP and NTproBNP concentrations at 0 days of age were higher (20-fold for NTroBNP and 18-fold for BNP) compared to adult levels in a prior study²⁵ and both peptides immediately decrease after birth.^{26,27} Koch et al. reported that plasma concentration of BNP had sex related differences in the second decade of life,²⁸ and even in adult studies, both peptides were related with age and gender.^{29–32} Likewise, we found concentrations in both BNP and NTproBNP levels decreased until age 12, after which they began to increase at slightly different rates between males and females. The associations with the clinical variables were however robust after adjusting for these potential confounding effects. In addition, a recent report showed that the decreasing trend of NTproBNP was different from BNP.³³ This result was replicated in the presented work as well. Therefore, interpretation of NPs in the clinical setting should include consideration of age- and gender-related differences.

Several limitations should be mentioned. First, as previously described, of the 88 patients, all patients survived, and did not receive lung transplantation. Therefore, we could not evaluate the predictive value of both peptides due to the low number of events. The present study was an observational cohort study from a single center, therefore, clinical findings regarding the prognostic impact of these NPs remain to be confirmed in a larger multi-site study. Second, there was heterogeneity in the study population because our patients included a variety of associated forms of PAH. Third, the patients were individually treated with various vasodilator therapies according to their conditions. Despite these limitations, we found that both biomarkers can be noninvasively assessed as promising tools for the evaluation of pediatric patients with PAH.

Conclusions

In conclusion, BNP and NTProBNP levels strongly correlated and were associated with clinical variables and hemodynamics in pediatric patients with variations in severity of PAH. Both concentrations were affected by age and gender. BNP tended to correlate better with catheterization variables. In a cross-sectional analysis, NTproBNP correlated with echocardiographic and exercise data better than BNP. NTproBNP showed less within patient variability over time, therefore NTproBNP can add additional information towards predicting these clinical measurements. There was a strong correlation between the BNP and NTproBNP levels and both biomarkers may be equally useful for evaluating disease severity, although due to different half-lives, one may be more appropriate in certain contexts. However, given the relatively low magnitude of the correlations, these biomarkers do not replace the clinical parameters, but may provide additional information. The measurements of both natriuretic peptides may serve as useful markers in the assessment of disease in patients with PAH.

Acknowledgments

This study was supported by the Jayden DeLuca Foundation, the Leah Bult Foundation, UL1 RR025780 Colorado Clinical Translational Science Institute, National Center for Research Resources, and National Institutes of Health.

References

1. Pfister R, Schneider CA. Natriuretic peptides bnp and nt-pro-bnp: Established laboratory markers in clinical practice or just perspectives? *Clin Chim Acta*. 2004; 349:25–38. [PubMed: 15469852]
2. Silver MA, Maisel A, Yancy CW, McCullough PA, Burnett JC Jr, Francis GS, Mehra MR, Peacock Wf, Fonarow G, Gibler WB, Morrow DA, Hollander J. Bnp consensus panel 2004: A clinical approach for the diagnostic, prognostic, screening, treatment monitoring, and therapeutic roles of natriuretic peptides in cardiovascular diseases. *Congest Heart Fail*. 2004; 10:1–30.
3. Alibay Y, Beauchet A, El Mahmoud R, Brun-Ney D, Alexandre JA, Benoit MO, Dubourg O, Aegerter P, Boileau C, Jondeau G, Puy H. Analytical correlation between plasma n-terminal pro-

- brain natriuretic peptide and brain natriuretic peptide in patients presenting with dyspnea. *Clin Biochem.* 2004; 37:933–936. [PubMed: 15369727]
4. Nakagawa O, Ogawa Y, Itoh H, Suga S, Komatsu Y, Kishimoto I, Nishino K, Yoshimasa T, Nakao K. Rapid transcriptional activation and early mrna turnover of brain natriuretic peptide in cardiocyte hypertrophy. Evidence for brain natriuretic peptide as an "emergency" cardiac hormone against ventricular overload. *J Clin Invest.* 1995; 96:1280–1287. [PubMed: 7657802]
 5. Clerico A, Emdin M. Diagnostic accuracy and prognostic relevance of the measurement of cardiac natriuretic peptides: A review. *Clin Chem.* 2004; 50:33–50. [PubMed: 14633912]
 6. Cowie MR, Jourdain P, Maisel A, Dahlstrom U, Follath F, Isnard R, Luchner A, McDonagh T, Mair J, Nieminen M, Francis G. Clinical applications of b-type natriuretic peptide (bnp) testing. *Eur Heart J.* 2003; 24:1710–1718. [PubMed: 14522565]
 7. Favilli S, Frenos S, Lasagni D, Frenos F, Pollini I, Bernini G, Arico M, Bini RM. The use of b-type natriuretic peptide in paediatric patients: A review of literature. *J Cardiovasc Med (Hagerstown).* 2009; 10:298–302. [PubMed: 19430339]
 8. Hartmann F, Packer M, Coats AJ, Fowler MB, Krum H, Mohacsi P, Rouleau JL, Tendera M, Castaigne A, Anker SD, Amann-Zalan I, Hoersch S, Katus HA. Prognostic impact of plasma n-terminal pro-brain natriuretic peptide in severe chronic congestive heart failure: A substudy of the carvedilol prospective randomized cumulative survival (copernicus) trial. *Circulation.* 2004; 110:1780–1786. [PubMed: 15381643]
 9. Mair J, Hammerer-Lercher A, Puschendorf B. The impact of cardiac natriuretic peptide determination on the diagnosis and management of heart failure. *Clin Chem Lab Med.* 2001; 39:571–588. [PubMed: 11522102]
 10. Tsutamoto T, Sakai H, Nishiyama K, Tanaka T, Fujii M, Yamamoto T, Horie M. Direct comparison of transcardiac increase in brain natriuretic peptide (bnp) and n-terminal probnp and prognosis in patients with chronic heart failure. *Circ J.* 2007; 71:1873–1878. [PubMed: 18037739]
 11. Leuchte HH, Holzapfel M, Baumgartner RA, Neurohr C, Vogeser M, Behr J. Characterization of brain natriuretic peptide in long-term follow-up of pulmonary arterial hypertension. *Chest.* 2005; 128:2368–2374. [PubMed: 16236896]
 12. Bernus A, Wagner BD, Accurso F, Doran A, Kaess H, Ivy DD. Brain natriuretic peptide levels in managing pediatric patients with pulmonary arterial hypertension. *Chest.* 2009; 135:745–751. [PubMed: 18849405]
 13. Lammers AE, Hislop AA, Haworth SG. Prognostic value of b-type natriuretic peptide in children with pulmonary hypertension. *Int J Cardiol.* 2009; 135:21–26. [PubMed: 18599134]
 14. Nagaya N, Nishikimi T, Uematsu M, Satoh T, Kyotani S, Sakamaki F, Kakishita M, Fukushima K, Okano Y, Nakanishi N, Miyatake K, Kangawa K. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation.* 2000; 102:865–870. [PubMed: 10952954]
 15. Fijalkowska A, Kurzyna M, Torbicki A, Szewczyk G, Florczyk M, Pruszczyk P, Szturmowicz M. Serum n-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. *Chest.* 2006; 129:1313–1321. [PubMed: 16685024]
 16. Van Albada ME, Loot FG, Fokkema R, Roofthoof MT, Berger RM. Biological serum markers in the management of pediatric pulmonary arterial hypertension. *Pediatr Res.* 2008; 63:321–327. [PubMed: 18287971]
 17. van Loon RL, Roofthoof MT, Delhaas T, van Osch-Gevers M, ten Harkel AD, Strengers JL, Backx A, Hillege HL, Berger RM. Outcome of pediatric patients with pulmonary arterial hypertension in the era of new medical therapies. *Am J Cardiol.* 2010; 106:117–124. [PubMed: 20609658]
 18. Thiebaut R, Jacqmin-Gadda H, Chene G, Leport C, Commenges D. Bivariate linear mixed models using sas proc mixed. *Comput Methods Programs Biomed.* 2002; 69:249–256. [PubMed: 12204452]
 19. Nagaya N, Nishikimi T, Okano Y, Uematsu M, Satoh T, Kyotani S, Kuribayashi S, Hamada S, Kakishita M, Nakanishi N, Takamiya M, Kunieda T, Matsuo H, Kangawa K. Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension. *J Am Coll Cardiol.* 1998; 31:202–208. [PubMed: 9426041]

20. McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, Mathier MA, McGoon MD, Park MH, Rosenson RS, Rubin LJ, Tapson VF, Varga J, Harrington RA, Anderson JL, Bates ER, Bridges CR, Eisenberg MJ, Ferrari VA, Grines CL, Hlatky MA, Jacobs AK, Kaul S, Lichtenberg RC, Moliterno DJ, Mukherjee D, Pohost GM, Schofield RS, Shubrooks SJ, Stein JH, Tracy CM, Weitz HH, Wesley DJ. Accf/aha 2009 expert consensus document on pulmonary hypertension: A report of the american college of cardiology foundation task force on expert consensus documents and the american heart association: Developed in collaboration with the american college of chest physicians, american thoracic society, inc., the pulmonary hypertension association. *Circulation*. 2009; 119:2250–2294. [PubMed: 19332472]
21. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *N Engl J Med*. 1998; 339:321–328. [PubMed: 9682046]
22. Hall C. Essential biochemistry and physiology of (nt-pro)bnp. *Eur J Heart Fail*. 2004; 6:257–260. [PubMed: 14987573]
23. Yeo KT, Wu AH, Apple FS, Kroll MH, Christenson RH, Lewandrowski KB, Sedor FA, Butch AW. Multicenter evaluation of the roche nt-probnp assay and comparison to the biosite triage bnp assay. *Clin Chim Acta*. 2003; 338:107–115. [PubMed: 14637274]
24. Leuchte HH, El Nounou M, Tuerpe JC, Hartmann B, Baumgartner RA, Vogeser M, Muehling O, Behr J. N-terminal pro-brain natriuretic peptide and renal insufficiency as predictors of mortality in pulmonary hypertension. *Chest*. 2007; 131:402–409. [PubMed: 17296640]
25. Soldin SJ, Soldin OP, Boyajian AJ, Taskier MS. Pediatric brain natriuretic peptide and n-terminal pro-brain natriuretic peptide reference intervals. *Clin Chim Acta*. 2006; 366:304–308. [PubMed: 16360137]
26. Mir TS, Laux R, Hellwege HH, Liedke B, Heinze C, von Buelow H, Laer S, Weil J. Plasma concentrations of aminoterminal pro atrial natriuretic peptide and aminoterminal pro brain natriuretic peptide in healthy neonates: Marked and rapid increase after birth. *Pediatrics*. 2003; 112:896–899. [PubMed: 14523183]
27. Nir A, Bar-Oz B, Perles Z, Brooks R, Korach A, Rein AJ. N-terminal pro-b-type natriuretic peptide: Reference plasma levels from birth to adolescence. Elevated levels at birth and in infants and children with heart diseases. *Acta Paediatr*. 2004; 93:603–607. [PubMed: 15174780]
28. Koch A, Singer H. Normal values of b type natriuretic peptide in infants, children, and adolescents. *Heart*. 2003; 89:875–878. [PubMed: 12860862]
29. Loke I, Squire IB, Davies JE, Ng LL. Reference ranges for natriuretic peptides for diagnostic use are dependent on age, gender and heart rate. *Eur J Heart Fail*. 2003; 5:599–606. [PubMed: 14607197]
30. Maisel AS, Clopton P, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AH, Steg G, Westheim A, Knudsen CW, Perez A, Kazanegra R, Bhalla V, Herrmann HC, Aumont MC, McCullough PA. Impact of age, race, and sex on the ability of b-type natriuretic peptide to aid in the emergency diagnosis of heart failure: Results from the breathing not properly (bnp) multinational study. *Am Heart J*. 2004; 147:1078–1084. [PubMed: 15199359]
31. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. Plasma brain natriuretic peptide concentration: Impact of age and gender. *J Am Coll Cardiol*. 2002; 40:976–982. [PubMed: 12225726]
32. Wang TJ, Larson MG, Levy D, Leip EP, Benjamin EJ, Wilson PW, Sutherland P, Omland T, Vasan RS. Impact of age and sex on plasma natriuretic peptide levels in healthy adults. *Am J Cardiol*. 2002; 90:254–258. [PubMed: 12127613]
33. Koch AM, Rauh M, Zink S, Singer H. Decreasing ratio of plasma n-terminal pro-b-type natriuretic peptide and b-type natriuretic peptide according to age. *Acta Paediatr*. 2006; 95:805–809. [PubMed: 16801175]

Figure 1. Concentrations for BNP (Blue) and NTproBNP (green) versus age

The general trend for males (solid line) and females (dashed line) are displayed separately using a scatterplot smoother. (236 samples in 88 patients)

* BNP; B-type natriuretic peptide, NTproBNP; amino-terminal fragment B-type natriuretic peptide

Figure 2. Scatterplot displaying the cross-sectional relationship between BNP and NTproBNP
The relationship between NP marker values is displayed; the diagonal line corresponds to the line of equivalence. The majority of the points lie below this line showing that the measured values of BNP were lower than the NTproBNP measurements obtained from the same sample. (88 samples in 88 patients)

Figure 3. The t-statistics corresponding to BNP and NTproBNP testing the association with each of the clinical variables estimated from serially collected measurements

NTproBNP had larger t-values for 6MWD, TR velocity, peak VO₂ and VE/VCO₂ compared to BNP, suggesting a better association.

* 6MWD; 6 minute walking distance, mPAP; mean pulmonary arterial pressure, mSAP; mean systemic arterial pressure, mRAP; mean right atrial pressure, PVRI; pulmonary vascular resistance index, CI; cardiac index, TR; tricuspid regurgitation, LVFS; left ventricular fractional shortening, Peak VO₂; peak oxygen consumption, VE/VCO₂; ratio of minute ventilation to carbon dioxide output

Figure 4. Correlations between serial TR velocity and natriuretic peptides measurements
 BNP and NTproBNP were significantly associated with TR velocity ($slope=34 \text{ (m/s)/(log pg/ml)}$, $p<0.01$, $slope=40.0 \text{ (m/s)/(log pg/ml)}$, $p<0.01$, respectively). However, there was no statistical difference between NTproBNP and BNP slopes. (214 samples in 69 patients)

Table 1

Patient demographics and clinical measurements

Patient demographics (patient's number = 88)	number (%)
Female	46 (52)
Idiopathic PAH	31 (35)
Congenital heart disease	39 (44)
Chronic lung disease	13 (16)
Clotting disease	4 (4)
Connective tissue disease	1 (1)
Sample characteristics (sample number = 263)	median (IQR)
Age (years)	10 (5 – 15)
Median (range) of blood samples pre patients	2 (1 – 9)
Catheterization variables (catheterization number = 86)	median (IQR)
mPAP (mm Hg)	38 (27 – 54)
Pulmonary Capillary Wedge Pressure (mm Hg)	8 (7 – 10)
mRAP (mmHg)	6 (4 – 8)
CI (L/min/m ²)	4.0 (3.3 – 5.3)
PVRI (units × m ²)	7.5 (4.2 – 12.3)
PVR/SVR	0.5 (0.3 – 0.9)
mPAP/mSAP	0.6 (0.5 – 0.9)
Follow-up time (months)	13.3 (0.2 – 34.0)
Adverse outcomes	number (%)
Expired	0
Transplantation	0
Initiation of IV prostanoids	3 (3.4)

* PAH; pulmonary arterial hypertension, mPAP; mean pulmonary arterial pressure, mRAP; mean right atrial, CI; cardiac index, PVRI; pulmonary vascular resistance index, SVR; systemic vascular resistance, mSAP; mean systemic arterial pressure

Table 2

Cross-sectional associations between NPs and clinical variables

Clinical variables (patient number)	Sensitivity Analysis			
	BNP <i>r</i> (<i>p</i> -value)	NT-proBNP <i>r</i> (<i>p</i> -value)	(patient number)	BNP <i>r</i> (<i>p</i> -value)
6MWD (41)	-0.32 (0.04)	-0.49 (<0.01)	(19)	-0.41 (0.08)
mPAP (47)	0.34 (0.02)	0.28 (0.06)	(39)	0.37 (0.02)
mPAP/mSAP (46)	0.40 (<0.01)	0.45 (<0.01)	(38)	0.42 (<0.01)
mRAP (45)	0.48 (<0.01)	0.48 (<0.01)	(38)	0.51 (<0.01)
PVRI (46)	0.30 (0.04)	0.23 (0.12)	(38)	0.23 (0.16)
CI (41)	-0.22 (0.16)	-0.01 (0.93)	(34)	-0.02 (0.90)
TR velocity (69)	0.36 (<0.01)	0.41 (<0.01)	(36)	0.34 (0.04)
LVFS (48)	0.33 (0.02)	0.19 (0.20)	(25)	0.54 (0.01)
RVDD (55)	-0.01 (0.97)	-0.02 (0.87)	(29)	-0.14 (0.46)
WHO (36)	0.32 (0.06)	0.35 (0.04)	(10)	0.06 (0.87)
Peak VO2 (20)	-0.29 (0.21)	-0.41 (0.07)	(10)	-0.09 (0.81)
VE/VCO2 (19)	0.11 (0.67)	0.30 (0.21)	(10)	-0.05 (0.89)
				-0.22 (0.53)

The sensitivity analysis restricts correlation estimates to measurements collected on the same day. Gray cells indicate statistically significant values

* 6MWD; 6 minute walking distance, TR; tricuspid regurgitation, LVFS; left ventricular fractional shortening, RVDD; right ventricular diastolic dimension by echocardiography, Peak VO2; peak oxygen consumption, VE/VCO2; ratio of minute ventilation to carbon dioxide output

Table 3

Associations between BNP markers and clinical variables accounting for repeated measures using all patient data.

Clinical variables (number of samples)	BNP (99 %CL)	NTproBNP (99 %CL)
6MWD (meters) (137)	−49.0 (−88.1 , −9.9)	−49.7 (−84.0 , −15.4)
mPAP (mmHg) (86)	12.9 (3.1 , 22.6)	9.0 (1.23 , 16.9)
mPAP/mSAP (84)	0.24 (0.08 , 0.40)	0.20 (0.07 , 0.32)
mRAP (mmHg) (83)	3.0 (1.3 , 4.7)	2.5 (1.2 , 3.8)
PVRI (unitsxm ²) (84)	4.5 (0.4 , 8.7)	3.4 (0.1 , 6.6)
CI (l/min/m ²) (74)	−0.55 (−1.8 , 0.7)	−0.26 (−1.2 , 0.7)
TR (m/s) (214)	0.34 (0.07 , 0.60)	0.40 (0.17 , 0.62)
FS (%) (170)	4.0 (0.01 , 8.1)	3.2 (−0.2 , 6.6)
Peak VO ₂ (ml/min/kg) (64)	−3.2 (−11.7 , 5.3)	−4.8 (−11.3 , 1.6)
VE/VCO ₂ (60)	1.8 (−5.4 , 8.9)	4.8 (−0.5 , 10.1)

The slope and the 99% CL are displayed. Gray cells indicate statistically significant value.

*
CL; confidence limits