Original Research

Correlation of Plasma B-Type Natriuretic Peptide with Shunt Volume in Children with Congenital Heart Disease Involving Left-to-Right Shunt

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Introduction: Concentrations of B-type natriuretic peptide (BNP) are recognised as a reliable marker of ventricular dysfunction in adults. In this study, plasma levels of BNP were determined in children with congenital heart disease (CHD) involving a left-to-right shunt, and were correlated with the shunt volume.

Methods: Seventy-six children (38 boys/38 girls, mean age 22.4 months) with CHD (Group A: 31 with atrial septal defect [ASD], 23 with ventricular septal defect [VSD], 8 with ASD and VSD, 14 with patent *ductus arteriosus* [PDA]) and 34 healthy children (group B) were studied. BNP was measured by chemiluminescent microparticle immunoassay in all children. The amount of shunt (the ratio of pulmonary blood flow/systemic blood flow: Qp/Qs) was measured using Doppler velocimetry and two-dimensional echocardiography. A haemodynamically significant left-to-right shunt was defined as Qp/Qs>1.5. Correlations were evaluated between all patient groups and healthy subjects and BNP was compared with echocardiographic data reflecting right and left ventricle volume overload.

Results: Thirty-four children of group A had Qp/Qs>1.5 (group A1) and 42 Qp/Qs<1.5 (group A2). BNP levels were higher in group A1 than group A2 (p=0.015), while there were no significant differences in BNP between group A2 and group B (p=0.79). BNP 24.4 pg/ml was determined as the cut-off point to identify patients with Qp/Qs>1.5. BNP values were similar among patients with ASD and VSD, but they were significantly higher in patients with PDA. BNP was positively correlated with Qp/Qs (r=0.59, p<0.001), and with the pulmonary artery velocity (r=0.27) and gradient (r=0.49), while there was a negative correlation with ejection fraction (r=-0.14). BNP levels were significantly higher in 10 infants with clinical signs of heart failure (p=0.025).

Conclusion: These results, which are consistent with previous reports, suggest a possible role of BNP as an early diagnostic marker of the significance of shunt in children with CHD.

B-type natriuretic peptide (BNP) is a cardiac hormone that is secreted mainly in the ventricles in response to increased wall stress. Released into the circulation, this peptide stimulates natriuresis and diuresis, and inhibits the renin–angiotensin–aldosterone system.[1-3]

In recent years, plasma levels of BNP have attracted increasing interest as markers of ventricular dysfunction.[4,5] Indeed, measurement of plasma BNP concentrations has been shown to be useful in the diagnosis, risk stratification, and management of adult patients with congestive

heart failure.[6-9] Moreover, BNP may be useful in various other conditions in adults, such as hypertrophic cardiomyopathy,[10] left ventricular (LV) remodelling after myocardial infarction, arrhythmogenic right ventricular (RV) dysplasia,[11] while finally it may be a strong predictor of mortality in adults with congenital heart disease (CHD).[12] It is worth noting that the number of adult patients with CHD is steadily increasing.[13]

Sporadic reports have described changes in BNP levels in children with familial hypertrophic cardiomyopathy, Kawasaki disease, renal hypertension, sleep-disordered breathing and anthracycline-induced cardiac damage.[14] BNP has also attracted interest as a screening tool for significant patent *ductus arteriosus* (PDA) in premature neonates, especially in cases where Doppler echocardiography is not easily available.[15]

However, in contrast to adults, the role of BNP concentrations in the evaluation and management of children with CHD, not only before but also after surgical repair, is much less clear. Only a few studies in the literature[16-18] refer to the evaluation of BNP in patients with ASD and VSD, the most remarkable being that of Ozhan et al.[17] The present study, which also included patients with PDA, was designed to determine plasma levels of BNP in children and infants with CHD involving a left-to-right shunt and to evaluate the relationship between BNP levels and the shunt volume.

Methods

Patients

As an experimental group (group A), we studied 76 children and infants (38 males, 38 females) suffering from CHD with a left-to-right shunt, aged from 1 month to 14 years (mean age 22.94 months). Neonates were excluded, as there is a general consensus that BNP levels are higher after birth and decrease rapidly during the first days of life.[19-21] As a model of LV volume overload we evaluated 23 patients with ventricular septal defect (VSD) and 14 with PDA, while as a model of RV volume overload, we included 31 patients with atrial septal defect (ASD). Another 8 patients had both ASD and VSD. All children in group A were evaluated before intervention (surgery or therapeutic cardiac catheterisation).

As a control group (group B) we studied 34 healthy children and infants who were free of congenital or other heart disease (16 males, 8 females), aged from 1 month to 14 years (mean age 23.04 months). None of these subjects had suffered birth asphyxia. The children who were included in the study did not differ statistically in terms of sex (p=0.275), age (p=0.081), or weight (p=0.05) Moreover, all subjects were free of acute illness (fever, infections, water and electrolyte imbalance, neoplasia, liver disease, or renal disease). They were not on drug treatment and were not receiving intravenous infusions. Physical examination and laboratory testing were normal.

All procedures were carried out in accordance with the Declaration of Helsinki 1975, revised 1983. The parents of the children were given full written information about the study and consented to their participation. This study was approved by the ethical committee of the Medical Faculty of Aristotle University of Thessaloniki.

For each child, a clinical examination, an electrocardiogram, blood sampling, and a transthoracic echocardiogram were performed on the day of enrolment.

Blood sampling and assay for BNP

Blood samples were collected after the echocardiogram had been recorded. Initially, a routine blood analysis was performed in all subjects to rule out organic lesions, infections, and electrolytic disorders. Blood (1.5 ml) was drawn from a peripheral vein and collected in plastic tubes to which disodium ethylenediamine tetraacetic acid (EDTA) and aprotinin (500 U/ml) were added. The tubes were immediately taken to the laboratory, centrifuged at 4°C for separation of the plasma, and the plasma was then frozen and stored at -70°C until analysis, which was performed within 3 months after the sampling. Plasma BNP levels were measured using a commercially available chemiluminescent microparticle immunoassay (CMIA) on the Architect i System.

Echocardiographic evaluation

A haemodynamic evaluation was performed using echo in all children. All children underwent a complete two-dimensional transthoracic echocardiographic and Doppler study in the left lateral decubitus position from multiple windows. All participants were typically supine and quiet during the exam. Infants who kept crying were anesthetized with chloral hydrate. Studies were performed using standard techniques, involving subxiphoid, precordial, apical,

and suprasternal measurements, with a Vivid 3 echocardiograph and a 5 MHz transducer. All echocardiographic studies were performed by expert echocardiographers who were blinded to the results of the BNP measurements.

LV function and size were estimated from the M-mode tracing in parasternal long-axis view. Ejection fraction (EF) was used as the principle marker of LV systolic function. Pulmonary to systemic flow ratio (Qp/Qs) was measured non-invasively using Doppler velocimetry and two-dimensional echocardiography. A Qp/Qs ratio >1.5 was considered to indicate the presence of a significant shunt.[22] Using Doppler velocimetry, we calculated the velocity and the peak Doppler gradient in the pulmonary artery.

Statistical analysis

All calculations were performed using the statistical package JMP 8.0 (SAS Inst., Cary NC, USA). Since the Shapiro–Wilk test revealed that almost all values followed log normal distributions, we used parametric methods. All data were expressed as mean value ± standard deviation. A value of $p < 0.05$ was considered significant. Lack of significance is indicated as p: NS (not significant). In particular, comparison of the BNP levels between patients and healthy subjects, and between children with CHD and Qp/Qs>1.5 and those with CHD and Qp/Qs<1.5, was carried out using one-way ANOVA with Tukey *post hoc* comparisons. Correlation of the BNP levels with haemodynamic parameters (Qp/Qs, pulmonary systolic volume, systemic systolic volume, tricuspid valve E/A ratio, EF, pulmonary artery velocity and gradient) was determined by Pearson's correlation coefficient. Comparison of plasma BNP in the patients with signs of congestive heart failure and those without was made using the t-test. Finally, in order to determine the cut-off point with the highest accuracy for plasma BNP in the identification of patients with a significant shunt, receiver operating characteristics curves were generated (ROC analysis).

Results

In the 34 healthy subjects, the mean BNP concentration was 12.753 ± 4.217 pg/ml (interquartile range 10 to 22.9 pg/ml). In the 31 patients with ASD the mean BNP concentration was 28.613 ± 29.317 (interquartile range 10 to 123.2 pg/ml); in the 23 patients with VSD it was 30.026 ± 31.341 (range 10 to 154 pg/ml);
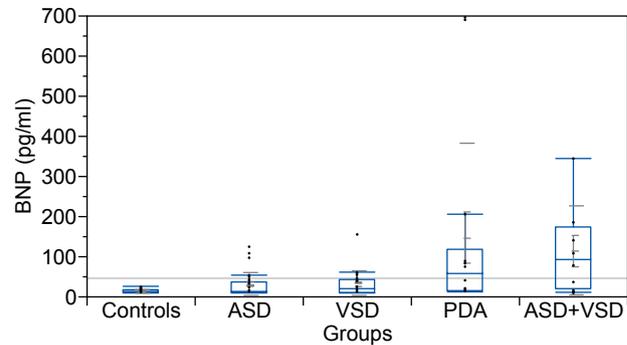

Figure 1. Levels of B-type natriuretic peptide (BNP) in the groups. ASD – atrial septal defect; VSD – ventricular septal defect; PDA – patent *ductus arteriosus*.

in the 14 with PDA it was 144.607 ± 238.031 (range 10 to 695.9); and in the 8 patients with both ASD and VSD it was 114 ± 111.684 (range 10 to 344.8 pg/ml). One-way ANOVA revealed that there were significant differences in BNP levels among these five groups ($p < 0.001$, F4,105=6.79; Figure 1).

In particular, the BNP levels were higher in subjects with CHD than in healthy subjects ($p < 0.001$). There were no significant differences in BNP levels between controls and patients with ASD, controls and patients with VSD, or patients with ASD and patients with VSD, while the BNP values were significantly higher in patients with PDA in comparison to all other groups. Moreover, the BNP levels did not differ according to sex ($p = 0.218$, $F_{2,107} = 3.41$) between all these groups.

Of the 76 children with CHD (group A), 34 had haemodynamically significant left-to-right shunts (Group A_1) and 42 had haemodynamically insignificant shunts (Group A_2). It is worth noting that the echocardiographic evaluation did not reveal patients with pulmonary hypertension (PH) in group A_1. Mean plasma BNP levels were higher in group A_1 than in group A_2 (99.84 ± 164.04 pg/ml vs. 26.65 ± 37.39 pg/ml, $p = 0.015$, [t]=2.5). Using one-way ANOVA to compare these two groups with controls (group B), we demonstrated that BNP levels were significantly higher in group A_1 ($p = 0.0007$, $F_{2,107} = 8.58$), while there was no significant difference in BNP levels between group A_2 patients and controls ($p = 0.79$; Figure 2).

Furthermore, in group A there was a positive and powerful linear correlation between BNP and the significance of the shunt (Qp/Qs) ($r = 0.59$, $p < 0.001$), and the pulmonary artery velocity ($r = 0.27$, $p = 0.017$) and gradient ($r = 0.49$, $p < 0.001$). In contrast, there
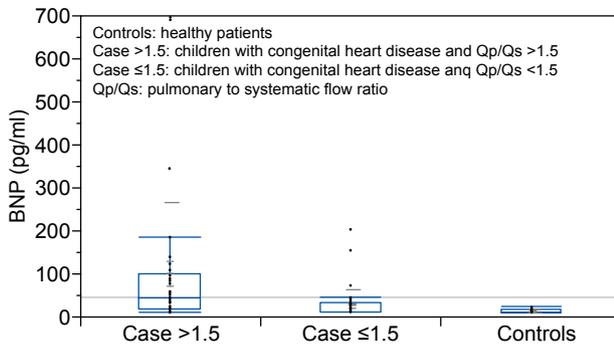
Figure 2. Relation between levels of B-type natriuretic peptide (BNP) and groups divided according to a haemodynamic variable (pulmonary to systemic flow ratio, Qp/Qs).

was a negative linear correlation with EF (r=-0.14, p: NS) and the tricuspid valve E/A ratio (r=-0.035, p: NS; Figure 3).

In patients with ASD (RV volume overload), BNP levels were significantly correlated with Qp/Qs (r=0.62, p<0.001) and pulmonary systolic volume (r=0.36, p=0.04), but not with systemic systolic volume (r=-0.13, p: NS). In patients with PDA (LV volume overload), too, the BNP levels were significantly correlated with Qp/Qs (r=0.84, p<0.001), pulmonary systolic volume (r=0.79, p<0.001), but not with systemic systolic volume (r=0.04, p: NS). In contrast, in patients with VSD (LV volume overload), the BNP levels were significantly correlated with systemic systolic volume (r=0.71, p=0.04), but not with Qp/Qs (r=0.28, p: NS) or pulmonary systolic volume (r=-0.27, p: NS).

According to ROC analysis, plasma BNP 24.4 pg/ml was determined as the cut-off point for identifying patients with Qp/Qs>1.5. At 24.4 pg/ml, plasma BNP had a sensitivity of 70.59% and a specificity of 82.89%. When the magnitude of the shunt was 2

for Qp/Qs, the cut-off point for BNP was 31.6 pg/ml (sensitivity 78.26%, specificity 83.91%). In patients with ASD and VSD these cut-off values were 22.2 pg/ml (sensitivity 84.62%, specificity 86.54%) and 19 pg/ml (sensitivity 83.33%, specificity 80.39%), respectively.

Finally, 10 patients of group A fulfilled the clinical criteria of congestive heart failure due to left-to-right shunt, but none of them had received medical treatment for this condition at the time of the study. Using the t-test, we revealed that BNP levels (mean 241.36 ± 254.32 pg/ml) were significantly higher in these infants, compared with patients of group A without heart failure (p=0.025, [t] =2.68).

Discussion

The present study indicates that plasma BNP can reflect pressure and volume loads in the pulmonary artery and right ventricle and may be helpful in the evaluation of infants and children with ASD, VSD, and PDA in cases where invasive measurements of heart function are not available. In fact, we found a significant positive correlation between the plasma concentration of BNP and the Qp/Qs evaluation, reflecting the shunt size of CHD, and the volume load in the pulmonary artery and right ventricle. There was also a strong correlation between BNP and the velocity and gradient in the pulmonary artery measured echocardiographically, reflecting the pressure load in the pulmonary artery and right ventricle. These findings are consistent with previous reports on BNP that showed a positive correlation between plasma BNP and Qp/Qs in children with different types of CHD, including ASD and VSD.[16-18] However, in these latter three studies, the paediatric patient groups were
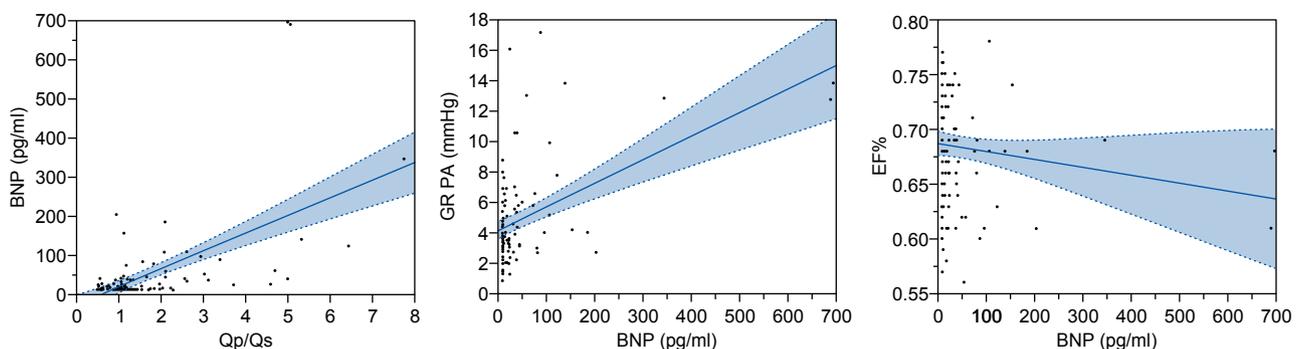

Figure 3. Bivariate fit of B-type natriuretic peptide (BNP, pg/ml) with pulmonary to systemic flow ratio (Qp/Qs), pulmonary artery gradient (GR PA, mmHg) and ejection fraction (EF).

limited in number and heterogeneous.[23] Our study extends their results, since it also included patients with PDA.

As expected, the LV function was normal in the population of our study, as left-to-right shunts in paediatric patients initially lead to RV or LV volume overload prior to the emergence of ventricular dysfunction.[18] In children with CHD and reduced LV systolic function, BNP levels have been found to be elevated.[24] A negative correlation between plasma BNP levels and LV systolic function has also been described in paediatric patients with CHD.[25,26] We also found a negative correlation between LV EF and plasma BNP levels, a result which is in accord with the above studies.

Children with CHD may progress to develop heart failure. In these patients, clinical signs of heart failure are associated with increased levels of certain biomarkers.[26-28] In our study, we made a similar observation. Despite the small number of our children who had clinical signs of heart failure, their mean plasma BNP level (241.36 pg/ml) was significantly higher compared with patients without congestive heart failure.

Plasma levels of BNP were higher in children with CHD than in those without. The higher plasma levels in children with CHD than in healthy children indicate that BNP reflects a significant load on the heart. The observation of our study, that plasma BNP levels are elevated in patients with CHD and Qp/Qs>1.5 compared to those with CHD and Qp/Qs <1.5, probably reflects the mechanism of BNP secretion by the ventricles when they are under haemodynamic stress. Indeed, BNP is released by the ventricular myocytes in response to LV volume overload as a result of significant left-to-right shunt.[29] On the other hand, we found that BNP values did not differ between patients with insignificant shunts and those without heart disease. Thus, we might conclude, extending the findings of Davlouros et al,[15] that elevated plasma BNP levels in patients with ASD, VSD, or PDA imply the existence of significant volume loading, not only in neonates but also in older children with CHD involving a left-to-right shunt. Therefore, plasma BNP may have a role in quantifying the severity of CHD with left-to-right shunt.

The accuracy of BNP for predicting the presence and the significance of shunt in all patients was also acceptable. Indeed, when we examined the relationship between the Qp/Qs ratio and BNP levels in all patients, we found that a Qp/Qs ratio of 1.5 cor-

responded to a BNP level of approximately 24.4 pg/ml. Likewise, a Qp/Qs ratio of 2.0 corresponded to a BNP level of 31.6 pg/ml. BNP<24 pg/ml cannot exclude pathology, but implies the absence of a significant left-to-right shunt. Our study suggests that BNP values >24 pg/ml in patients with a suspicion of a shunt should indicate echocardiographic evaluation, as other studies with slightly lower cut-off values have also suggested.[17]

A Qp/Qs ratio of 1.5 to 2.0 is generally considered an indication for surgical correction of a shunt lesion.[30,31] Accordingly, plasma BNP levels could potentially be used as a non-invasive method of indicating the need for surgical repair. Our findings indicate that a BNP level of 24.4 to 31.6 pg/ml is useful as an indication for surgery in ASD, VSD, and PDA patients; these results, in line with previous studies, make BNP a strong candidate as a predictive tool for early intervention.[16,17] It is worth noting that a very recent study revealed that plasma BNP levels are elevated in children with heart disease before surgical treatment.[32]

BNP concentration has been shown to be associated with the presence of a haemodynamically significant PDA in premature infants and to decrease to normal levels with ductal closure.[33-36] In our study, which included older children with PDA, we found that patients with PDA and either a significant or a non-significant shunt always had significantly higher plasma BNP levels than all other groups (not only controls, but also patients with ASD and VSD). In contrast, there were no significant differences in BNP levels among controls and ASD and VSD patients. A possible explanation for this is that PDA causes a more significant haemodynamic burden than ASD and VSD.

In conclusion, this study suggests that measurement of BNP levels in blood samples is a useful non-invasive indicator of volume overload, of both the left and the right ventricle, in infants and children with ASD, VSD or PDA, and could thus be a useful clinical tool in managing children with CHD involving left-to-right shunt. Measurement of plasma BNP concentration is rapid, inexpensive, and widely available. This measurement cannot replace cardiac imaging, but in the future it may provide information complementary to echocardiography for the evaluation of cardiac function and clinical status. The results should therefore be interpreted in the context of similar data from other studies and prospectively more accurate methods are needed in this field.

Study limitations

The main limitations of this study were the limited number of patients and the wide age range, as BNP levels may be different in puberty. Another limitation was the use of echocardiographic data for comparison instead of data from catheterisation, which is the normal standard. The results should therefore be interpreted in the context of similar data from other studies.

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