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The Role of N-Terminal Pro-B-Type Natriuretic Peptide in the Diagnosis of Congestive Heart Failure in Children —Correlation With the Heart Failure Score and Comparison With B-Type Natriuretic Peptide—

(小児のうっ血性心不全診断におけるN-末端Pro-B型Na利尿ペプチドの役割—心不全評点との相関及びB型Na利尿ペプチドとの比較—)

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The Role of N-Terminal Pro-B-Type Natriuretic Peptide in the Diagnosis of Congestive Heart Failure in Children

– Correlation With the Heart Failure Score and Comparison With B-Type Natriuretic Peptide –

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Background: Both B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) are useful biomarkers for the assessment of congestive heart failure (CHF) in adults. The purpose of this study was to determine whether BNP and NT-proBNP levels could be used to stratify the severity of CHF in children.

Methods and Results: The study comprised 181 children with CHF and 232 healthy children aged from 4 months to 14 years who were categorized into CHF grades I, II, III and IV according to the modified Ross scoring system. The plasma BNP and serum NT-proBNP levels were significantly correlated with increasing CHF grades. The NT-proBNP levels were significantly different among the 4 CHF grades. However, only 2 significant differences were observed in the BNP levels between each CHF grade. NT-proBNP testing with cut-off points of >438 pg/ml (≥grade II), >1,678 pg/ml (≥grade III) and >7,734 pg/ml (grade IV) in the patients below 3 years of age, and >295 pg/ml (≥grade II), >1,545 pg/ml (≥grade III) and >3,617 pg/ml (grade IV) in those above 3 years of age was determined to be highly sensitive and specific by receiver operating characteristic analysis.

Conclusions: The blood levels of BNP and NT-proBNP therefore reflect the severity of CHF in children. In particular, NT-proBNP is a useful biomarker for evaluating CHF in children. (*Circ J* 2010; **74:** 998–1005)

Key Words: BNP; Children; Congenital heart disease; Congestive heart failure; NT-proBNP

B-type natriuretic peptides (BNPs) are released into the blood from myocardial cells in response to various kinds of stress on the heart. The BNP peptides exert various physiological functions such as diuretic action, vasodilation and myocardial remodeling.[1] The plasma level of BNP has been reported to increase in heart failure in adults[1,2] and even in children.[3]

In fact, several types of BNP molecules are present in the blood.[4] The N-terminal pro-BNP (NT-proBNP) is an N-terminal protein, originating from proBNP and is released from the myocardial cellular membrane when BNP is broken down by a protein known as furin.[5] NT-proBNP has no physiological function, and is excreted in its original form from the kidney.[6] Because of these characteristics, NT-proBNP has recently received much attention as a useful cardiac biomarker for evaluation of congestive heart failure (CHF) in place of BNP. Moreover, it has a longer half-life than BNP in the peripheral blood and it can also be measured in the plasma as well as in the serum[7,8] and is stable at normal temperatures, thus allowing for easy storage.

Children with congenital heart disease often suffer from heart failure resulting from abnormal hemodynamics, such as volume and pressure overload[3,9]; however, there are few reports on the use of NT-proBNP as a biomarker for heart failure in children.[10–12]

The present study established the reference range of the plasma BNP and serum NT-proBNP levels in healthy children and compared the blood levels of the two biomarkers with the clinical score based on symptoms for heart failure in children. Furthermore, the cut-off levels were determined to reveal the range of BNP and NT-proBNP levels predictive of the heart failure grades.

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Table 1. Scoring System for Grading CHF Infants and Children According to Ross,[26] Reithmann et al[27] and Mir et al[13]

	Score (points)		
	0	1	2
History			
Diaphoresis	Head only	Head and body during exercise	Head and body at rest
Tachypnea	Rare	Several times	Frequent
Physical examination			
Breathing	Normal	Retractions	Dyspnea
Respiratory rate (breaths/min)			
0–1 (years)	<50	50–60	>60
1–6 (years)	<35	35–45	>45
7–10 (years)	<25	25–35	>35
11–14 (years)	<18	18–28	>28
Heart rate (beats/min)			
0–1 (years)	<160	160–170	>170
1–6 (years)	<105	105–115	>115
7–10 (years)	<90	90–100	>100
11–14 (years)	<80	80–90	>90
Hepatomegaly (liver edge from right costal margin)	<2 cm	2–3 cm	>3 cm

Total score: 0–2=no CHF; 3–6=mild CHF; 7–9=moderate CHF; 10–12=severe CHF.
CHF, congestive heart failure.

Methods

Patients and Healthy Volunteers

From November 2005 to September 2008, we prospectively enrolled 413 clinically stable subjects, including 181 children with heart disease (CHF group) and 232 healthy volunteer children (healthy group). The age ranged from 4 months to 14 years. All children in the CHF group were admitted to the Department of Pediatrics of Asahikawa Medical College Hospital, Japan. The children with a wide variety of CHF severity on the day of the study were categorized according to a modified Ross scoring system of CHF signs (Table 1).[13] Each sign or symptom was graded on a scale of 0, 1, or 2 points according to the severity. The sum of points formed the clinical score (range, 0–12 points), with a higher score corresponding to more severe heart failure. There were 108 patients with asymptomatic CHF (grade I), 45 with mild CHF (grade II), 17 with moderate CHF (grade III) and 11 with severe CHF (grade IV) as shown in Table 2. Table 2 also lists the various types of cardiac lesions and medications currently taken by the children. The healthy group was enrolled at an affiliated general hospital when they were discharged after treatment for disease such as respiratory tract infection, asthma and epilepsy. No children with renal dysfunction were observed in either of the 2 groups. In particular, renal function is known to mature to the young adult level at around 3 years of age. Therefore, we sorted the subjects into 2 groups: those below 3 years of age and those 3 years of age or older. All subjects were fully informed about the procedures, risks and benefits of the study, and written informed consent was obtained from all subjects and their respective families before the study commenced. This study was also approved by the Ethics Committee of Asahikawa Medical College Hospital.

Measurement of Plasma BNP and Serum NT-ProBNP Concentrations

Blood samples were taken from the superior vena cava or an antecubital vein. The samples were withdrawn into plastic syringes and transferred to 2 chilled siliconized disposable tubes. The sample for the BNP assay was collected in EDTA-containing tubes and centrifuged within 1 h. The plasma and serum samples were immediately frozen at –80°C. All samples were thawed only once at the time of assay. The plasma BNP concentration was measured using a commercially available immunoassay kit (Shionoria BNP assay kit; Shionogi Ltd, Osaka, Japan). The serum NT-proBNP concentration was measured on an Elecsys 2010 analyzer with a chemiluminescent immunoassay kit (Roche Diagnostics; Mannheim, Germany). Further details are available elsewhere.[14]

Statistical Analysis

A least-squares regression line was fitted to both the BNP and NT-proBNP concentrations vs age plots for the healthy group. Spearman's rank correlation was calculated to assess the correlations between the data. The correlation between BNP and NT-proBNP with respect to the total distribution of the healthy and CHF groups combined was statistically examined using Pearson's correlation coefficient as there were no significant differences in fitting curves with a power function to the 2 variables between the healthy and CHF groups. A Kruskal-Wallis analysis of variance (ANOVA) was used to examine the overall differences among the healthy group and the 4 graded CHF groups because the variables of BNP and NT-proBNP were not always normally distributed. If the ANOVA findings were significant, then the differences between the groups were estimated using the Steel-Dwass test. A receiver operating characteristic (ROC) analysis was carried out to examine the diagnostic utility of BNP and NT-proBNP levels for distinguishing between the 4 successive grades of CHF in each age group. Differences were considered to be significant for all statistical analyses at a value of $P<0.05$.

Table 2. Characteristics of Healthy Children and Children With Heart Disease

Ross class	Healthy group	CHF group			
		I (no CHF)	II (mild CHF)	III (moderate CHF)	IV (severe CHF)
N	232	108	45	17	11
Age, median (years) (range)	3.3 (4 months–14.9years)	3.1 (4 months–14.6years)	1.3 (4 months–13.7years)	1.1 (4 months–10.7years)	0.8 (4 months–13.7 years)
<3.0 years (n)	98	48	32	15	7
≥3.0 years (n)	134	60	13	2	4
Sex (M/F)	134/98	63/45	25/20	8/9	8/3
Clinical score (mean±SD)	0.2±0.4	0.7±0.9	3.8±0.9	7.6±0.9	10.6±0.7
Medications					
Diuretic		28 (26%)	25 (56%)	14 (82%)	8 (73%)
ACE inhibitor/ARB		18 (17%)	7 (16%)	4 (24%)	4 (36%)
Digitalis		5 (5%)	2 (4%)	2 (12%)	2 (18%)
β-blocker		5 (5%)	3 (7%)	3 (18%)	0 (0%)
ET-blocker		5 (5%)	4 (9%)	3 (18%)	2 (18%)
Underlying heart diseases					
Congenital heart disease		92 (85%)	43 (96%)	16 (94%)	9 (82%)
Atrial septal defect (with pulmonary hypertension)		19 (0)	8 (1)	1 (1)	1 (1)
Ventricular septal defect		13	7	4	0
Double outlet right ventricle		8	3	1	2
Tetralogy of Fallot		5	6	0	0
Tricuspid valve atresia		6	6	0	0
Single ventricle		3	6	0	0
Atrioventricular septal defect		1	2	4	4
Transposition of the great arteries		7	0	2	1
Total anomalous pulmonary venous connection		4	3	3	0
Pulmonary atresia		2	6	1	0
Patent ductus arteriosus		7	0	0	0
Aortic valve stenosis		5	1	0	0
Coarctation of the aorta		4	0	0	0
Hypoplastic left heart syndrome		0	1	0	1
Miscellaneous		8	0	0	0
Acquired heart disease		16 (15%)	2 (4%)	1 (6%)	2 (18%)
Kawasaki disease with coronary lesion		12	1	0	1
Arrhythmia		4	0	0	1
Dilated cardiomyopathy		0	1	1	0

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; ET-blocker, endothelin-1 receptor blocker.

Results

Figures 1A,B show the relationship between both peptide levels and age in healthy children. The BNP levels were relatively constant with respect to age. However, the NT-proBNP levels slightly but significantly decreased with advancing age, whereas the range of NT-proBNP levels was broader in infants than in adolescents.

Figure 2 shows the relationship between individual NT-proBNP levels and the corresponding BNP levels in CHF children together with those in healthy children. The slope and intercept of the regression line for the CHF children were not significantly different from those for the healthy children. Therefore, for both healthy and CHF children, NT-proBNP levels were significantly correlated with the corresponding BNP levels, ie, $NT\text{-}proBNP=9.080\times BNP^{0.923}$ ($r=0.856$, $n=413$, $P<0.005$).

The correlation between both the BNP and NT-proBNP levels and the CHF grades is shown in Figures 3A,B. Although the BNP levels increased as the CHF grade increased in both the patients groups below 3 years of age and those above 3 years of age, respectively ($r=0.593$, $P<0.001$, $r=0.483$, $P<0.001$), significant differences were only detected between CHF grades I and II in the patients below 3 years of age and between CHF grades II and III in the patients above 3 years of age. In contrast, the NT-proBNP levels significantly increased in both the groups below 3 years of age and in those above 3 years of age, respectively ($r=0.655$, $P<0.001$, $r=0.476$, $P<0.001$). Significant differences were detected among the 4 CHF grades in the patients below 3 years of age and between CHF grades I and II, CHF grades II and III in the patients 3 years of age or older. However, no significant differences were observed between the healthy group and the CHF grade I group regarding both levels of BNP and NT-proBNP. The median (25^{th}–75^{th} percentiles) NT-proBNP levels of the patients with several CHF grades in the patients below 3 years of age and above 3 years of age are shown in Table 3. NT-proBNP levels in the healthy group were significantly different between the patients below 3 years of age and those above 3 years of age. NT-proBNP levels in

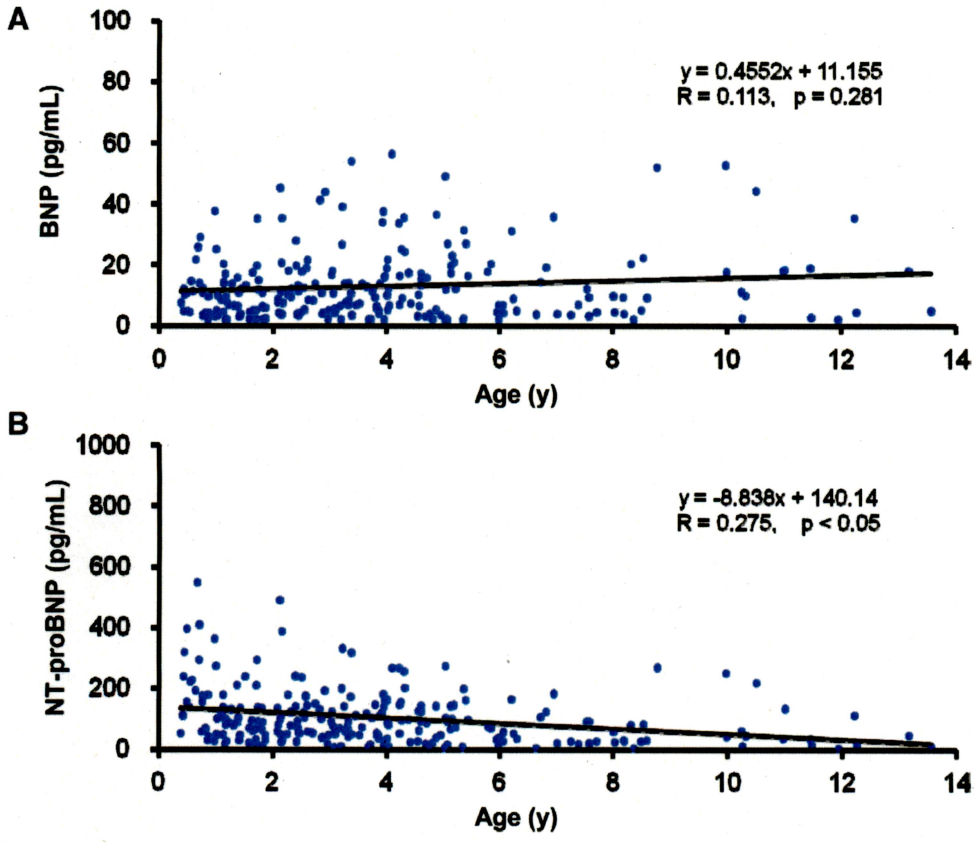

Figure 1. Relationship between the (**A**) BNP levels and (**B**) NT-proBNP levels and age in the healthy children (n=232). BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro-BNP.

CHF grade I were significantly different between the patients below 3 years of age and those above 3 years of age. However, no significant differences were observed in the patients below 3 years of age and those above 3 years of age regarding CHF grades II, III and IV.

The ROC curves for the BNP and NT-proBNP levels with respect to CHF grades II through IV are shown in **Figure 4**. The area under the ROC curve with respect to CHF grade II and over was 0.894 for BNP and 0.955 for NT-proBNP in the patients below 3 years of age (**Figure 4A-1**). To identify patients with CHF grade II and over, a BNP cut-off value of 31.2 pg/ml was 83.0% sensitive and 83.6% specific, and an NT-proBNP cut-off value of 438.4 pg/ml was 88.7% sensitive and 91.8% specific in the patients below 3 years of age. Similarly, the area under the ROC curve for CHF grade III and over (**Figure 4A-2**) and CHF grade IV (**Figure 4A-3**) was 0.860 and 0.837 for BNP, and 0.984 and 0.999 for NT-proBNP in the patients below 3 years of age, respectively. The area under the ROC curve with respect to CHF grade II and over was 0.964 for BNP and 0.986 for NT-proBNP in the patients above 3 years of age (**Figure 4B-1**). Similarly, the area under the ROC curve for CHF grade III and over (**Figure 4B-2**) and CHF grade IV (**Figure 4B-3**) was 0.994 and 0.987 for BNP, and 0.997 and 0.994 for NT-proBNP in the patients above 3 years of age, respectively. The cut-off values, sensitivity, and specificity are summarized in **Table 4**.

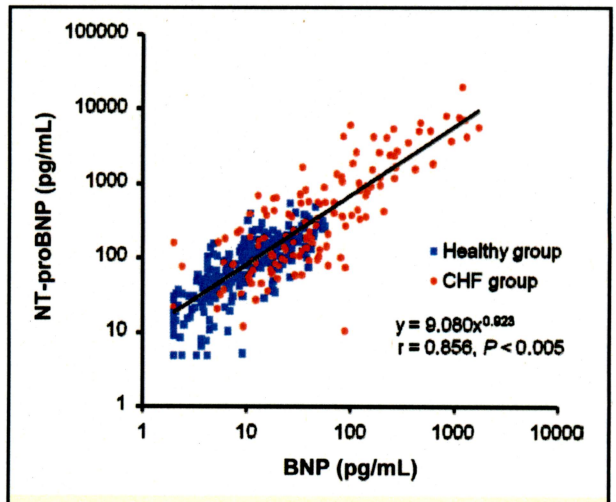

Figure 2. Relationship between the BNP and NT-proBNP levels in the healthy group (blue boxes, n=232) and CHF group (red circles, n=181) on logarithmic coordinates. BNP, B-type natriuretic peptide; CHF, congestive heart failure; NT-proBNP, N-terminal pro-BNP.

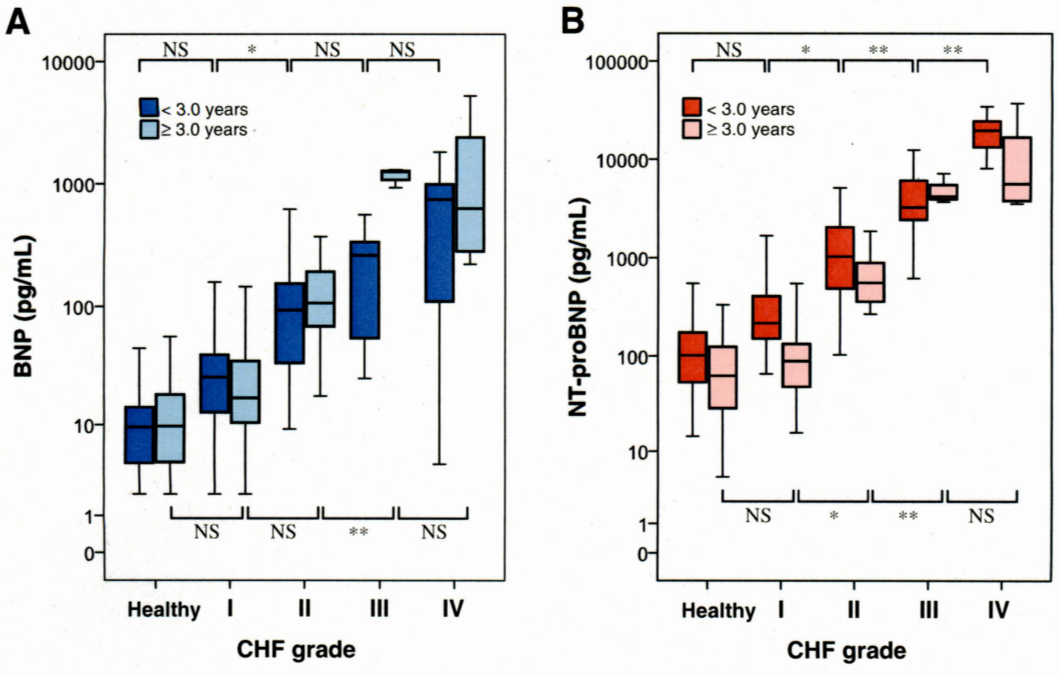

Figure 3. The (**A**) BNP and (**B**) NT-proBNP levels in the healthy group and 4 CHF grades on a logarithmic scale. In each of the CHF grades, the left boxes indicate below 3 years of age and the right boxes indicate 3 years of age or older, respectively. Bars represent the median, 5th, 25th, 75th and 95th percentiles. *P<0.05; **P<0.01. NS, not significant; BNP, B-type natriuretic peptide; CHF, congestive heart failure; NT-proBNP, N-terminal pro-BNP.

Table 3. The Median (25th–75th Percentiles) NT-ProBNP Levels of Patients With Several CHF Grades

	Healthy group	CHF group			
		I (no CHF)	II (mild CHF)	III (moderate CHF)	IV (severe CHF)
<3.0 years (4 months–3 years)	102.3 (54.3–170.5)	219.2 (153.9–398.0)	995.0 (519.2–1,565.0)	3,259.5 (2,483.0–5,950.5)	19,784.0 (13,949.5–24,642.0)
≥3.0 years (3–14 years)	63.2 (29.1–124.7)	89.4 (49.9–133.7)	561.1 (361.0–895.2)	4,217.0 (3,963.0–5,714.5)	5,926.5 (3,978.0–15,043.0)
Significant difference	<0.05	<0.05	NS	NS	NS

NT-ProBNP, N-terminal pro-B-type natriuretic peptide (BNP); NS, not significant. Other abbreviation see in Table 1.

Discussion

This study evaluated the relationship between both the BNP and NT-proBNP levels and the 4 grades of CHF severity in pediatric patients classified using a modified Ross score and in healthy children. As reported previously in adults, there was a significantly positive correlation between both the BNP and NT-proBNP levels and the CHF grades.

Recently, the determination of cardiac biomarkers, such as atrial natriuretic peptide (ANP) and BNP has been used for the clinical evaluation of heart failure.[15] In 2007, the American Association for Clinical Chemistry also advocated the use of BNP and NT-proBNP as beneficial biomarkers for evaluating heart failure and established guidelines for their use.[16] Because NT-proBNP has a longer half-life (70 min) than ANP (12 min) or BNP (15 min),[6] it is less influenced by stress on the heart just before the blood sampling. Furthermore, unlike BNP, blood samples for NT-proBNP measurement can be collected using the same containers for serum as those used for other biochemical tests. These factors are extremely beneficial in the pediatric setting because of the difficulty in keeping the patient quiet during blood sampling and the limited volume of blood that can be smoothly collected.

The BNP and NT-proBNP levels in healthy adults increase with advancing age[17,18] in part due to an age-related decline in renal function.[19] However, in healthy children, the BNP and NT-proBNP levels decrease with age from infancy to adolescence, except for neonates.[20–23] Since both the BNP and NT-proBNP levels show an abrupt increase immediately after birth followed by a gradual decrease to a level similar to that in adults over the subsequent 3 months,[20–23] children less than 4 months of age were excluded from this study.

There was a strong correlation between the BNP and NT-proBNP levels (r=0.856; **Figure 2**). Although BNP and NT-proBNP are derived from the same prohormone (proBNP molecule), the correlation coefficient could not be 1.0 for several reasons. First, the NT-proBNP levels showed a moderate decrease with age. Conversely, the BNP levels showed no tendency with age. Although the renal function of the

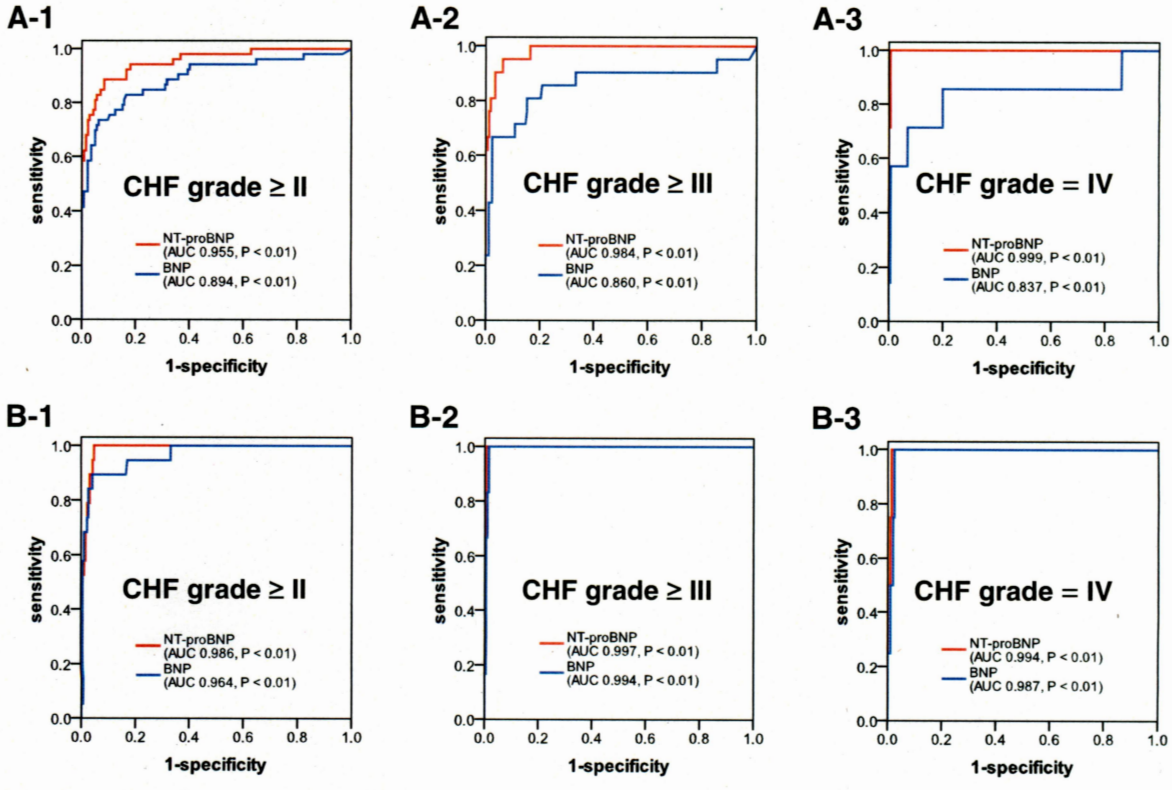

Figure 4. Receiver operating characteristics curves of the BNP and NT-proBNP levels: (**A-1**) CHF grade II and over, (**A-2**) CHF grade III and over, and (**A-3**) CHF grade IV in the patients below 3 years of age. (**B-1**) CHF grade II and over, (**B-2**) CHF grade III and over, and (**B-3**) CHF grade IV in the patients above 3 years of age. AUC, area under receiver operating characteristics curve; BNP, B-type natriuretic peptide; CHF, congestive heart failure; NT-proBNP, N-terminal pro-BNP.

Table 4. Diagnostic Accuracy of BNP and NT-ProBNP in Pediatric Patients With CHF

CHF grade	<3.0 years (4 months–3 years)			≥3.0 years (3–14 years)		
	CHF ≥ II	CHF ≥ III	CHF IV	CHF ≥ II	CHF ≥ III	CHF IV
BNP						
Cut-off point (pg/ml)	31.2	52.1	209.5	29.7	201.5	313.0
AUC	0.894	0.860	0.837	0.964	0.994	0.987
Sensitivity (%)	0.830	0.810	0.714	0.947	1.000	1.000
Specificity (%)	0.836	0.848	0.932	0.830	0.986	0.976
NT-pro BNP						
Cut-off point (pg/ml)	438.4	1,677.5	7,733.5	295.2	1,544.5	3,617.0
AUC	0.955	0.984	0.999	0.986	0.997	0.994
Sensitivity (%)	0.887	0.952	1.000	0.947	1.000	1.000
Specificity (%)	0.918	0.938	0.995	0.959	0.990	0.986

AUC, area under receiver operating characteristic curve. Other abbreviations see in Tables 1,3.

children in this study was normal, the excretion rate of NT-proBNP could be more susceptible to a lower glomerular filtration rate in younger children than that of BNP.[24,25] Second, the half-life of BNP is shorter than that of NT-proBNP, so the effect of stress on the heart during blood sampling may also have been a factor contributing to the variability of the BNP levels. Third, BNP is more unstable than NT-proBNP in vitro. It is also possible that the measurement results reflect the time lag between the time of specimen collection and measurement.

In adults, the severity of heart failure can be classified according to the widely used New York Heart Association (NYHA) classification of cardiac status; however, it is difficult to use this classification with children. In this regard, Ross et al[26] created a scale for assessing the severity of heart failure in children, but the scale is based on criteria that include the duration and amount of breast feeding, thus limiting its use. Accordingly, Reithmann et al[27] made revisions to the Ross scale to extend its application to children of all ages and formulated a clinical scoring system for heart fail-

ure symptoms. The current finding of a significant correlation between the NT-proBNP levels and the clinical heart failure score is entirely consistent with those of Mir and co-workers.[13] As a "gold" standard is yet to be established, it is considered reasonable to select a modified Ross scoring system as a "common" standard. Because the NT-proBNP level in adults has been reported to have a strong correlation with the NYHA classification of cardiac status,[28–30] the revised clinical score was used in place of such classification in younger children. The comparative figure shows a strong correlation between the CHF grade and both the BNP and NT-proBNP levels (**Figure 3**); the deterioration of the CHF grade is accompanied by a significant increase in both levels. However, the NT-proBNP levels were significantly different among all CHF grades, whereas the BNP levels were different only between grades I and II in the patients below 3 years of age. The NT-proBNP levels were significantly different between CHF grades I and II, and CHF grades II and III; however, the BNP levels were significantly different only between grades II and III in the patients between above 3 years of age. Moreover, no IQRs overlapped each other among all CHF grades in NT-proBNP, whereas all IQRs did in BNP in the patients between below 3 years of age. Consequently, NT-proBNP was determined to have a higher accuracy than BNP as a biomarker for identifying each CHF grade.

A cut-off level was determined for each grade by making an ROC curve to predict the CHF grade based on the blood peptide level (**Figure 4**). This approach could be useful because scaling the clinical score tends to be rather complicated in actual clinical practice. When the cut-off level was compared with that reported for adults,[28] the level of NT-proBNP was 438.4 pg/ml in CHF grade II in the patients below 3 years of age and 295.2 pg/ml in those above 3 years of age, which were slightly higher. However, considering the increase in the levels with decreasing age, the observed level thus seemed to be reasonable. Moreover, NT-proBNP had a larger AUC than BNP in each CHF grade, which is therefore considered to be additional evidence that NT-proBNP is a more specific and sensitive biomarker for identifying the CHF grades.

Delicate treatment is therefore required in clinical practice involving congenital heart diseases in children, thus making it extremely important to clearly identify the severity of heart failure. Accordingly, determining the serum NT-proBNP level in CHF may potentially make a significant contribution to cardiovascular management in children.

Study Limitations

The clinical factors that influence BNP and NT-proBNP levels are age, sex, renal function, obesity and the assay methods used.[17–19,31–33] In particular, renal function is known to mature to the young adult level at around 3 years of age. It is possible that the NT-proBNP level was influenced by the renal function when the NT-proBNP level was low. It is therefore possible that NT-proBNP does not precisely reflect heart failure in patients below 3 years of age. In the future, we should therefore stratify the renal function more carefully using renal functional markers such as sistatin C. The second limitation of this study was the small number of patients with a higher grade of CHF. The third limitation of this study was related to the fact that there is still no gold standard for a symptom-based heart failure scoring system in children. We think that it is very difficult to make the perfect scoring system in patients ranging in age from infants to teenagers. The final limitation of this study is due to the fact that we could not clarify whether the modified Ross's score correlated with cardiac function (eg, echocardiographies).

Conclusion

This study clearly demonstrated that the blood levels of BNP and NT-proBNP reflect the severity of CHF in children. In particular, NT-proBNP is considered to be a useful biomarker to evaluate CHF in children.

Disclosures

We hereby confirm that there are no known conflicts of interest associated with this research and there has been no significant financial support for this work that could have influenced its outcome.

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