

N-Terminal Pro B-Type Natriuretic Peptide Levels in Infants and Children with Acute Non-Cardiac Diseases

Inbar Nevo MD¹, Mati Erlichman MD⁴, Nurit Algur MSc³ and Amiram Nir MD²

¹Hebrew University-Hadassah Medical School, Jerusalem, Israel

²Department of Pediatric Cardiology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

³Clinical Laboratory and ⁴Department of Pediatrics, Shaare Zedek Medical Center, Jerusalem, Israel

ABSTRACT: **Background:** Cardiac patients express elevated levels of B-type natriuretic peptide and the amino terminal segment of its prohormone (NT-proBNP). However, there are non-cardiac causes of NT-proBNP level elevation.

Objectives: To determine the upper limit of NT-proBNP for pediatric patients with acute non-cardiac disease.

Methods: We compared NT-proBNP concentrations in children with acute non-cardiac, mostly febrile disease with concentrations in children with acute cardiac disease and in healthy children. We used the Student *t*-test and Mann-Whitney test for group comparisons, and Pearson's and Spearman's correlation coefficients to test relationships between variables.

Results: In 138 patients with acute non-cardiac diseases (mean age 3.7 years, 53% male), median NT-proBNP concentration was 162 pg/ml, upper limit (95% percentile) 1049 pg/ml. The level did not vary significantly by disease category; was negatively correlated with weight, weight percentile, age and hemoglobin level; and positively correlated with creatinine level. Multivariate analysis showed weight to be the only factor influencing NT-proBNP level. Levels were higher in children with acute non-cardiac diseases versus healthy children (median 88 pg/ml, $P < 0.001$, $n = 59$), and lower than levels in patients with acute cardiac disease (median 29,986 pg/ml, $P < 0.001$, $n = 30$). Receiver operating characteristic analysis showed good NT-proBNP performance for differentiation between children with acute cardiac versus non-cardiac disease (area under the curve 0.958), at a cutoff of 415 pg/ml.

Conclusions: NT-proBNP levels are higher in children with acute non-cardiac diseases than in healthy children, but lower than in children with acute cardiac disease. NT-proBNP negatively correlated with weight and weight percentile.

IMAJ 2011; 13: 420-424

KEY WORDS: natriuretic peptides, infants, children, heart disease, acute illness, fever

to counter the effects of the renin-angiotensin-aldosterone system [1-3]. The cardiac members of this family are the atrial natriuretic peptide and the B-type natriuretic peptide. ANP is secreted primarily from the cardiac atria in response to increased left or right atrial pressure as well as volume loads, whereas BNP is secreted primarily from the ventricles in response to increased left or right ventricular pressure and volume loads.

Bioactive BNP is comprised of 32 amino acids. The precursor molecule is cleaved into bioactive BNP and an N-terminal fragment, N-terminal proBNP, which consists of 76 amino acids, and has no bioactivity. Both cleaved fragments are released to the circulation [4]. Both BNP and NT-proBNP have emerged as important markers for heart failure in adults [5-7]. Plasma BNP and NT-proBNP levels are elevated in adult patients with left ventricular dysfunction [1], ischemic heart disease, diastolic dysfunction [5], and hypertrophic cardiomyopathy [8]. BNP levels correlate with the severity of left ventricular dysfunction and congestive heart failure, as well as prognosis [9]. Peptide levels were also found to be elevated in right heart failure [10].

In recent years, kits for the measurement of BNP and NT-proBNP have become available for commercial laboratory systems, enabling tests of BNP and NT-proBNP levels to serve as markers for heart disease in everyday practice. In adults, BNP and NT-proBNP are now used to identify heart failure in patients with symptoms suggestive of heart disease [6], to monitor the effectiveness of heart failure therapy [7], and to predict prognosis [1].

Data on the natriuretic peptides in pediatric heart disease are limited but suggest that peptides also have potential as markers for heart disease in children. Normal values have been established, showing elevated levels after birth with a gradual decrease after the neonatal period. However, a few studies and unpublished observations suggest that peptide levels may also be elevated in acute infections [11] and other non-cardiac disease [12] in children. It is thus important to determine char-

ANP = atrial natriuretic peptide
BNP = B-type natriuretic peptide
NT-proBNP = N-terminal proBNP

The natriuretic peptides are a family of bioactive peptides that affect sodium and water balance [1]. They induce natriuresis, diuresis and vasodilatation, and specifically act

acteristic levels in these conditions to avoid misinterpretation of high natriuretic peptide levels as a sign of heart disease. The purpose of the present study was to determine NT-proBNP levels in infants and children with acute non-cardiac diseases.

PATIENTS AND METHODS

This prospective study was performed according to the requirements of our Institutional Review Board, and informed consent was obtained from all parents. The study population comprised children who presented to the emergency department of Shaare Zedek Medical Center, a community hospital. Inclusion criteria for enrollment were age 2 months to 14 years, no known heart disease, and no sign of cardiac pathology in the patient's history or on physical examination. Children with known heart disease or hemodynamic instability were excluded from the non-cardiac disease study group.

Levels of NT-proBNP in age-matched healthy children from a previous study by our group [13] and in children with known acute heart disease whose NT-proBNP levels were tested during routine clinical evaluation were used as a basis for comparison. Blood was taken via peripheral venous puncture as part of the routine clinical evaluation in all patients. When blood was drawn for clinical studies, an additional 3 ml sample was collected in a test tube containing ethylenediaminetetraacetic acid (EDTA). Plasma (1 ml) was separated and stored at -20°C.

NT-proBNP was measured by a sandwich electrochemiluminescence immunoassay (Elecys 1010, Roche Diagnostics, Mannheim, Germany, <http://www.roche.com/diagnostics/>) within 2 weeks following plasma separation. The assay range was 5–35,000 pg/ml, with coefficient of variation < 6.5% at all NT-proBNP concentrations. The NT-proBNP kit is not affected by icterus (bilirubin < 35 mg/dl), hemolysis (hemoglobin < 1.4 g/dl), or lipemia (triglycerides < 4000 mg/dl).

STATISTICAL ANALYSIS

Since the data were not normally distributed, analysis was performed using log transformed NT-proBNP values and non-parametric statistical techniques. The Student *t*-test and Mann-Whitney test were used for group comparisons. Pearson's and Spearman's correlation coefficients were used to test the strength of relationships between variables. Receiver operating characteristic curve analysis was carried out to investigate the diagnostic performance of NT-proBNP for identifying infants with heart disease. Linear regression analysis was performed for detecting factors influencing NT-proBNP levels.

RESULTS

Data from 138 patients with non-cardiac disease (mean age 3.7 years, median 2 years; 52.7% male), 30 with cardiac disease (mean age 4 years, median age 1.7 years; 64% male), and 59

healthy children who were not diagnosed with either non-cardiac or cardiac disease (mean age 5.8 years, median age 3.7 years; 59% male) were included in the analysis.

Diagnoses in 138 patients with non-cardiac disease included gastroenteritis (n=15, 10.9%), dehydration (n=22, 15.9%), respiratory tract infection (n=23, 17%), fever due to viral disease (n=32, 23%), and fever due to bacterial disease (n=24, 17%). The remaining children in this group (n=22, 15.9%) presented with other problems such as abdominal pain and arthralgia and were defined as "other."

Diagnoses in 30 patients with cardiac disease included acute left ventricular dysfunction (6, 20%), myocarditis or acute cardiomyopathy (15, 50%), cardiomyopathy secondary to tachycardia or sepsis (1, 3.3%), acute rheumatic fever (3, 10%), Kawasaki's disease (3, 10%), endocarditis (1, 3.3%), and pericardial tamponade (1, 3.3%).

NT-proBNP LEVELS

Plasma NT-proBNP levels in patients with non-cardiac diseases are presented in Table 1. NT-proBNP concentrations were significantly higher (*P* < 0.001) in patients for each non-cardiac subgroup compared to healthy infants and children, but there were no differences between the subgroups. NT-proBNP levels for the non-cardiac group as a whole were thus used for further calculations.

NT-proBNP levels in all patients with non-cardiac diseases compared to levels in healthy children and patients with

Table 1. Comparison between NT-proBNP concentrations in subgroups of patients with non-cardiac disease

Diagnosis	N	NT-proBNP (pg/ml)		P (non-cardiac disease vs. healthy children)
		Mean ± SD	Median	
Gastroenteritis	15	413 ± 627	192	0.081
Dehydration	22	278 ± 376	173.5	0.048
Respiratory tract infection	23	314 ± 452	184	0.041
Fever (viral)	32	385 ± 526	243.5	0.006
Fever (bacterial)	24	202 ± 187	134.5	0.024
Other	22	164 ± 151	164.5	0.102
Healthy	59	109 ± 95	88	NA

NA = not available

Table 2. Comparison between NT-proBNP concentrations in healthy children and in pediatric patients with acute non-cardiac and acute cardiac diseases

	NT-proBNP (pg/ml)			P value vs. non-cardiac disease
	Mean ± SD	Median	Range	
Non-cardiac disease	292 ± 417	162	5–2891	NA
Healthy	109 ± 95	88	5–391	< 0.001
Acute cardiac disease	50,229 ± 70,127	29,986	65–288,000	< 0.001

NA = not available

Figure 1. Comparison between NT-proBNP concentrations in healthy children and pediatric patients with acute non-cardiac (sick) and acute cardiac diseases (cardiac). NT-proBNP concentrations are displayed on a logarithmic scale. Bars represent median, 5th, 25th, 75th, and 95th percentiles. Dots represent individual outliers. $P < 0.001$ for children with acute non-cardiac disease vs. those with acute cardiac disease and in good health

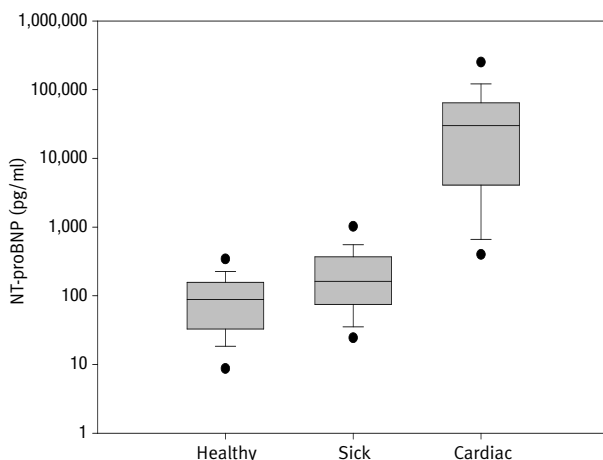
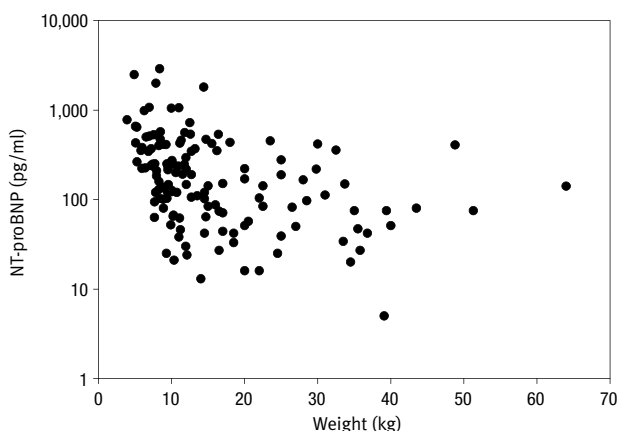


Figure 2. Correlation between NT-proBNP level and weight. NT-proBNP concentrations are displayed on a logarithmic scale



acute heart disease are presented in Table 2 and Figure 1. The median plasma NT-proBNP level in non-cardiac patients was 162 pg/ml, range 5–2891 pg/ml; the 95th percentile was 1049 pg/ml. This was significantly higher than the level in healthy children (median 88 pg/ml, $P < 0.001$, $n=59$), and significantly lower than the level in pediatric cardiac patients (median 29,986 pg/ml, $P < 0.001$, $n=30$). The one patient in the acute cardiac disease group with a low NT-proBNP level (65 pg/ml) was diagnosed with Kawasaki's disease. On echocardiogram, this patient had normal cardiac function and normal diameter but "echo-bright" proximal coronary arteries, suggesting some degree of coronary arteritis.

CORRELATION OF NT-proBNP TO CLINICAL AND LABORATORY FACTORS

In patients with acute non-cardiac diseases, a negative correlation ($P < 0.001$) was found for NT-proBNP level compared with weight ($r = -0.37$) [Figure 2], weight percentile, age ($r = -0.32$), and hemoglobin level ($r = -0.32$). A positive correlation was found with creatinine levels ($r = 0.31$). Linear regression analysis showed that patient weight ($P = 0.01$) was the only clinical factor significantly influencing NT-proBNP level; the correlation to age did not remain significant ($P = 0.277$). There was no significant correlation between NT-proBNP level and patient gender. There was also no correlation between NT-proBNP level and body temperature, heart rate, systolic or diastolic blood pressure, or oxygen saturation. Similarly, no correlation was found with white blood cell count, platelet number, serum sodium, potassium, liver function tests, glucose, or lactate dehydrogenase levels. Whether the patient was hospitalized or discharged from the emergency department was also not correlated with NT-proBNP level.

Receiver operating characteristic curve analysis showed that NT-proBNP is not a good test to differentiate between infants with non-cardiac disease and healthy infants, with an area under the curve of only 0.69. However, when comparing NT-proBNP levels between pediatric patients with non-cardiac disease and those with acute cardiac disease, the AUC was 0.958 with a cut-off of 415 pg/ml. Sensitivity was 96%, specificity 79.7%, positive predictive value 50% and negative predictive value 99.1%.

DISCUSSION

NT-proBNP is an important biomarker for heart disease in adults [1-6] and children [14]. For routine use in clinical practice, expected values in various clinical situations should be established. In this study we determined NT-proBNP concentrations in infants and children with common acute non-cardiac diseases. The upper limit in patients with acute non-cardiac disease was 1049 pg/ml, significantly higher than the published upper limit for normal children [15] but significantly lower than levels for pediatric patients with acute cardiac diseases. We found a median NT-proBNP level of 162 pg/ml, which is less than the 241 pg/ml median reported by Hammerer-Lercher et al. [16] in a study involving a heterogeneous group of pediatric patients under 3 years old with acute non-cardiac disease. This difference may be due to the higher average age of patients in our study.

The important message from our data is that a child with acute non-cardiac disease can have NT-proBNP up to 1000 pg/ml and should not be suspected of having acute heart disease if no other signs or symptoms support this diagnosis. Furthermore, our data suggest that a child with acute disease

AUC = area under the curve

whose NT-proBNP is below 400 pg/ml is very unlikely to have acute heart disease (negative predictive value 99%). However, this last statement should be taken with caution, as the number of patients with acute heart disease in our study was small and patients with acute Kawasaki's disease [17] or rheumatic fever with mild cardiac involvement may have low NT-proBNP.

Elevated peptide levels can be a result of increased production, decreased clearance, or both. The peptide may be of cardiac origin or may be secreted by other tissues. There are a number of possible reasons for the higher levels of NT-proBNP levels in children with acute non-cardiac illnesses. While cardiac insult may occur as a part of the disease process in these patients, this appears unlikely as they show no other evidence of cardiac damage. Fever is associated with increased cardiac output. In a previous study [12] we reported high NT-proBNP levels in patients with sepsis and normal left ventricular systolic function. In acute non-cardiac disease we noted a moderate elevation in NT-proBNP levels, yet the elevation was not as high as levels reported in sepsis [12]. Stress hormones, known to induce secretion of the natriuretic peptides, are elevated during febrile illness. Pro-inflammatory cytokines have been reported to induce BNP synthesis and probably stimulate peptide release in a state of acute disease [11].

Another novel and interesting observation in this study was the negative correlation between the NT-proBNP levels and body weight. In adults, BNP and NT-proBNP were reported to be lower in overweight and obese heart failure patients compared with lean patients [18,19]. Horwich and colleagues [18] found that obesity was associated with a greater than sixfold increase in the odds of having low BNP. Similarly, Krauser et al. [19] showed nearly identical suppression of BNP and NT-proBNP with increasing body mass index.

Several mechanisms could potentially explain the inverse association between natriuretic peptides and BMI. First, the natriuretic peptide clearance receptor has been isolated in human adipose tissue [20], and both elevated NPR-C expression and increased secretion of neutral endopeptidases have been demonstrated in obese patients [21]. These findings suggest that natriuretic peptide clearance may be increased in obese patients, which could partly explain the impact of BMI on BNP plasma levels. However, peripheral elimination of NT-proBNP is not mediated by NPR-C activity or neutral endopeptidase degradation. Consequently, impaired synthesis and release of NT-proBNP from myocytes in obese subjects may also contribute to reduced circulating levels of this peptide in patients with higher BMI. To the best of our knowledge, correlation with body weight and weight percentiles have not been reported in the pediatric age group. This phenomenon can, at least partially, explain the decrease in peptide levels with age.

BMI = body mass index

NPR-C = natriuretic peptide clearance receptor

LIMITATIONS

Our study has a number of limitations. The population of pediatric patients with non-cardiac disease is relatively small. Echocardiogram was not performed in subjects in the study group. However, it is extremely unlikely that a child with no history and no signs or symptoms of cardiac disease would have asymptomatic cardiac dysfunction, a very rare disease in childhood. Since acute heart disease is rare in infants and children, we obtained data for patients with acute cardiac disease from our pediatric cardiology clinical practice. The number of patients with acute cardiac disease is small and it is possible that patients with mild acute cardiac diseases could have NT-proBNP levels lower than reported here.

CONCLUSIONS

NT-proBNP levels were elevated in infants and children with acute non-cardiac diseases but were lower than the levels in children with acute cardiac disease. Levels of NT-proBNP that distinguish between cardiac disease and common acute non-cardiac disease were determined. NT-proBNP levels were correlated negatively with weight and weight percentile.

Acknowledgments

Prof. Nir reports receiving travel expenses from Roche Diagnostics. During earlier research, Roche donated kits for post-studies. The authors have no other potential conflicts of interest to report. The authors wish to thank Shifra Fraifeld, a Research Coordinator in the Department of Pediatric Cardiology of Hadassah-Hebrew University Medical Center, for her superb editorial assistance in the preparation of this manuscript.

Corresponding author:

Dr. A. Nir

Dept. of Pediatric Cardiology, Hadassah-Hebrew University Medical Center, P.O. Box 12000, Jerusalem 91120, Israel

Phone: (972-2) 677-8132

Fax: (972-2) 643-4520

email: amiramn@ekmd.huji.ac.il

References

1. 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 (7): 571-88.
2. Baughman KL. B-type natriuretic peptide – a window to the heart. *N Engl J Med* 2002; 347 (3): 158-9.
3. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *N Engl J Med* 1998; 339 (5): 321-8.
4. Gotze JP, Kastrup J. Plasma pro-brain natriuretic peptides are strong biochemical markers in clinical cardiology. *Scand J Clin Lab Invest Suppl* 2001; 234: 47-51.
5. Lubien E, DeMaria A, Krishnaswamy P, et al. Utility of B-natriuretic peptide in detecting diastolic dysfunction: comparison with Doppler velocity recordings. *Circulation* 2002; 105 (5): 595-601.
6. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl J Med* 2002; 347 (3): 161-7.

7. Troughton RW, Frampton CM, Yandle TG, Espiner EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet* 2000; 355 (9210): 1126-30.
8. Nakamura T, Sakamoto K, Yamano T, et al. Increased plasma brain natriuretic peptide level as a guide for silent myocardial ischemia in patients with non-obstructive hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2002; 39 (10): 1657-63.
9. Abassi Z, Karram T, Ellaham S, Winaver J, Hoffman A. Implications of the natriuretic peptide system in the pathogenesis of heart failure: diagnostic and therapeutic importance. *Pharmacol Ther* 2004; 102 (3): 223-41.
10. Kucher N, Printzen G, Goldhaber SZ. Prognostic role of brain natriuretic peptide in acute pulmonary embolism. *Circulation* 2003; 107 (20): 2545-7.
11. Cowie MR, Mendez GF. BNP and congestive heart failure. *Prog Cardiovasc Dis* 2002; 44 (4): 293-321.
12. Fried I, Bar-Oz B, Algur N, et al. Comparison of N-terminal pro-B-type natriuretic peptide levels in critically ill children with sepsis versus acute left ventricular dysfunction. *Pediatrics* 2006; 118 (4): e1165-8.
13. 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 (5): 603-7.
14. Nasser N, Perles Z, Rein AJ, Nir A. NT-proBNP as a marker for persistent cardiac disease in children with history of dilated cardiomyopathy and myocarditis. *Pediatr Cardiol* 2006; 27 (1): 87-90.
15. Nir A, Lindinger A, Rauh M, 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.
16. Hammerer-Lercher A, Geiger R, Mair J, et al. Utility of N-terminal pro-B-type natriuretic peptide to differentiate cardiac diseases from noncardiac diseases in young pediatric patients. *Clin Chem* 2006; 52 (7): 1415-19.
17. Dahdah N, Siles A, Fournier A, et al. Natriuretic peptide as an adjunctive diagnostic test in the acute phase of Kawasaki disease. *Pediatr Cardiol* 2009; 30 (6): 810-17.
18. Horwich TB, Hamilton MA, Fonarow GC. B-type natriuretic peptide levels in obese patients with advanced heart failure. *J Am Coll Cardiol* 2006; 47 (1): 85-90.
19. Krauser DG, Lloyd-Jones DM, Chae CU, et al. Effect of body mass index on natriuretic peptide levels in patients with acute congestive heart failure: a proBNP investigation of dyspnea in the Emergency Department (PRIDE) substudy. *Am Heart J* 2005; 149 (4): 744-50.
20. Sarzani R, Dessi-Fulgheri P, Paci VM, Espinosa E, Rappelli A. Expression of natriuretic peptide receptors in human adipose and other tissues. *J Endocrinol Invest* 1996; 19 (9): 581-5.
21. Dessi-Fulgheri P, Sarzani R, Tamburrini P, et al. Plasma atrial natriuretic peptide and natriuretic peptide receptor gene expression in adipose tissue of normotensive and hypertensive obese patients. *J Hypertens* 1997; 15 (12 Pt 2): 1695-9.