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

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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. Multivariant 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.

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

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 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
characteristic 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 (Elecsys 1010, Roche Diagnostics, Manheim, 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%), myocardiitis 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>
<thead>
<tr>
<th>Diagnosis</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>P (non-cardiac disease vs. healthy children)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastroenteritis</td>
<td>15</td>
<td>413 ± 627</td>
<td>192</td>
<td>0.081</td>
</tr>
<tr>
<td>Dehydration</td>
<td>22</td>
<td>278 ± 376</td>
<td>173.5</td>
<td>0.048</td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>23</td>
<td>314 ± 452</td>
<td>184</td>
<td>0.041</td>
</tr>
<tr>
<td>Fever (viral)</td>
<td>32</td>
<td>385 ± 526</td>
<td>243.5</td>
<td>0.006</td>
</tr>
<tr>
<td>Fever (bacterial)</td>
<td>24</td>
<td>202 ± 187</td>
<td>134.5</td>
<td>0.024</td>
</tr>
<tr>
<td>Other</td>
<td>22</td>
<td>164 ± 151</td>
<td>164.5</td>
<td>0.102</td>
</tr>
<tr>
<td>Healthy</td>
<td>59</td>
<td>109 ± 95</td>
<td>88</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not available

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

NA = not available
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 cutoff 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
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.

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.

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References


**Capsule**

**Targeting ROR-γt on Th17 cells by small molecules**

Two groups report on the development of small-molecule inhibitors of T helper type 17 (Th17) cells. The transcription factor retinoic acid receptor-related orphan nuclear receptor-γt (ROR-γt) is expressed in Th17 cells, is required for interleukin-17 (IL-17) production and is crucial in driving autoimmune disease. Littman et al. (Nature doi: 10.1038/nature09978) found that the clinically approved cardiac glycoside digoxin specifically inhibited ROR-γt. Derivatives of digoxin with reduced toxicity in human cells reduced IL-17 production by T cells and the severity of clinical symptoms in a mouse model of multiple sclerosis. Derivatives of the liver X receptor agonist T0901317 also inhibit ROR-γt, according to a study by Burris et al. (Nature doi: 10.1038/nature10075). Treatment of CD4+ T cells with the compound SR1001, which acts as an inverse agonist of both ROR-γt and ROR-α, also suppressed IL-17 expression and delayed the onset and clinical severity of disease in a mouse model of multiple sclerosis. Although several antibody-based strategies aimed at inhibiting IL-17 are being tested in clinical trials, this class of compounds provides a new means of targeting Th17 cells and treating autoimmune disease.

Eitan Israeli

**Capsule**

**Seeking the right pathway for losartan in Marfan syndrome**

Activation of transforming growth factor-β (TGFβ) signaling promotes the development of aortic aneurysms in the connective tissue disorder Marfan syndrome (MFS). Losartan, a drug that inhibits TGFβ signaling, is in clinical trials for this disorder. Like many cytokines, TGFβ activates multiple intracellular signaling pathways. In the context of aortic disease, TGFβ has been assumed to act through the “canonical” Smad pathway. Holm et al. (Science 2011; 311: 358) and Habashi et al. (p. 361) show that the “non-canonical” TGFβ pathway, which involves the signaling proteins ERK1/2, is the prominent driver of aortic disease in MFS mice and that it is this pathway through which losartan exerts its beneficial effects. Analysis of ERK1/2 activation status in MFS patients may help optimize losartan dosage, and drugs specifically targeting the non-canonical pathway may merit exploration as possible therapies for aortic aneurysms.

Eitan Israeli

“Everybody wants to save the earth; nobody wants to help mom do the dishes”

P.J. O’Rourke (born 1947), American writer and satirist