N-Terminal Pro-Brain Natriuretic Peptide as a Useful Diagnostic Marker of Acute Kawasaki Disease in Children

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Background: Serum N-terminal pro-brain natriuretic peptide (NTproBNP) is often elevated in patients with acute Kawasaki disease (KD), but the NTproBNP level in normal children is higher than in adults. Thus, characterization of the normal levels and cut-off values of NTproBNP according to age is warranted for proper diagnosis of acute KD in children.

Methods and Results: Six hundred and fifty-five patients aged 1 month–15 years (median, 2.9 years) were included. Patients were admitted to the NTT East Japan Sapporo Hospital between October 2007 and October 2011. Serum NTproBNP level was examined in 149 patients with KD (median, 2.1 years) and 506 control patients with acute infectious disease (median, 3.2 years). In the control group, a Z-score curve of NTproBNP was generated for each age group using least mean square-based methods. The Z-score distribution of KD patients was then compared with that of the control group. The specificity and sensitivity of NTproBNP for diagnosing acute KD were 97.8% and 47.0%, respectively, at Z-score >2.0. Additionally, simple cut-offs every 100 pg/ml according to age were established for more convenient use at the bedside.

Conclusions: The Z-score curve for NTproBNP in children was characterized. A Z-score >2.0 or the cut-off for children may be used to diagnose acute KD. (Circ J 2013; 77: 2097–2101)

Key Words: Children; Diagnosis; Kawasaki disease; Natriuretic peptide

Kawasaki disease (KD), also known as Kawasaki syndrome, lymph node syndrome, and mucocutaneous lymph node syndrome, is an autoimmune disease in which the medium-sized blood vessels throughout the body become inflamed.1,2 Although it has been thought that symptoms of KD are related to hyperactivation of the immune system triggered by infection with some microorganisms, the etiological agent remains unknown. Recently, susceptibility genes for KD have been identified in succession in studies utilizing a genome-wide approach.3–5

In the absence of a specific diagnostic test for KD, clinical criteria have been established to assist the physician in making the diagnosis. The diagnostic criteria for KD, based on the principal clinical findings, are (1) fever persisting ≥5 days (inclusive of cases in which the fever subsided before the fifth day in response to therapy); (2) bilateral conjunctival congestion; (3) changes of lips and oral cavity: reddening of lips, strawberry tongue, diffuse involvement of oral and pharyngeal mucosa; (4) polymorphous exanthema; (5) changes of peripheral extremities (initial stage, reddening of palms and soles, indurative edema; convalescent stage, membranous desquamation from fingertips); and (6) cervical lymphadenopathy. At least 5 items of (1–6) need to be satisfied for diagnosis of KD. Patients with 4 of the principal symptoms, however, can be diagnosed with KD when coronary aneurysm or dilatation is identified on 2-D echocardiography or coronary angiography.6

Kaneko et al reported that serum N-terminal pro-brain natriuretic peptide (NTproBNP) is elevated in KD, and that NTproBNP is useful for the prediction of risk of coronary arterial lesions (CAL) in KD.7 Other recent studies also suggested that this biomarker may be a useful diagnostic tool in children with atypical or early-phase KD. Serum NTproBNP is often elevated in acute KD, but normal NTproBNP varies with age in children. Thus, the diagnosis of acute KD requires an establishment of age-based cut-offs for NTproBNP in children. In studies involving blood measurements in pediatric patients, it is important to establish age-matched reference values. Some previous studies have already reported on the normal...
levels of NTproBNP according to age. In the present study, we generated normal curves for NTproBNP level, according to age, using statistically rigid least mean square (LMS)-based methods. We then examined the diagnostic power of NTproBNP for KD. Age-based standard and cut-off values for NTproBNP are a helpful diagnostic tool for pediatric heart failure.

Methods

Six hundred and fifty-five patients aged 1 month–15 years of age (median, 2.9 years) were included. Patients were admitted to the NTT East Japan Sapporo Hospital between October 2007 and October 2011. Serum NTproBNP level was measured in 149 patients with KD (median=2.1 years) and 506 control patients with other acute infectious disease (median=3.2 years). In the control group, almost 80% of them had febrile disease. Patients with cardiac disease and severe respiratory disease, for example asthma and respiratory syncytial virus disease, which induce pulmonary hypertension and right heart failure, were excluded. Infants <1 month of age had extremely high NTproBNP level and were excluded from this study.

This study was approved by the hospital ethics committee and written informed consent was obtained from the patients’ parents for participation.

Serum NTproBNP level was measured at the time of hospital admission via electrochemiluminescent immunoassay using a Cobas 6000 e601 (Roche Diagnostic, Tokyo, Japan). It can give accurate measurements with only 0.1 ml of serum. In the KD group, serum NTproBNP level was measured several times during hospitalization. Peak NTproBNP was then used to estimate the Z-score in KD patients. In the control group, we established a Z-score curve for serum NTproBNP level using the LMS method and program (lmsChartMaker light version 2.0; Figure 1). The LMS method determines the changing distribution using the median (M), coefficient of variation (S), and skewness (L), expressed as a Box-Cox power. We used these values to optimize the curves according to the levels of NTproBNP.

Table 1. Specificity and Sensitivity of Kawasaki Disease Diagnosis

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>Z-score 1.0</th>
<th>Z-score 2.0</th>
</tr>
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<tbody>
<tr>
<td>Specificity (%)</td>
<td>82.8</td>
<td>97.8</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>77.2</td>
<td>47.4</td>
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</table>

In Z-score 2.0 of N-terminal pro-brain natriuretic peptide or the determined cutoff values for bed side usage, diagnostic specificity was very high.

Figure 1. Z-score curves for the relationship between N-terminal pro-brain natriuretic peptide (NTproBNP) and age in control children. Z-score curves of 0 (median), ±1, and ±2 were plotted for children aged 1 month–15 years. Infants <1 month of age had extremely high NTproBNP level and were excluded from this study.

Figure 2. Z-score distribution of N-terminal pro-brain natriuretic peptide level in patients (red) with and (blue) without Kawasaki disease (KD). The majority of patients with a Z-score >2 presented with KD.

Figure 3. Receiver operating characteristic curve for Kawasaki disease (KD) diagnosis. The area under the curve was 0.85, indicating that N-terminal pro-brain natriuretic peptide level is a useful tool for KD diagnosis and for distinguishing KD from other acute diseases.
were excluded from this study.

There was a gradual decrease in NTproBNP with increasing age. The distribution of Z-scores in KD patients tended to be higher than that of control patients.

The specificity and sensitivity of NTproBNP were examined for Z-score cut-offs of +1.0 and +2.0. The specificity and sensitivity of NTproBNP for diagnosing acute KD were 82.8% and 77.2%, respectively, for Z-score = 1.0, and 97.8% and 47.4%, respectively, for Z-score = 2.0 (Table 1).

The present findings suggest that the cut-offs for NTproBNP for the diagnosis of acute KD appear to be optimum for a Z-score cut-off of 1.0, as determined via receiver operating characteristic curve analysis (Figure 3). But, because it is better to have higher specificity for diagnosing atypical or early-phase KD, we believe that setting the Z-score cut-off at 2.0 is better.

**Table 2.** NTproBNP Cut-Offs vs. Patient Age

<table>
<thead>
<tr>
<th>Patient age</th>
<th>NTproBNP cut-off (pg/ml)</th>
<th>Median (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–11 months</td>
<td>1,000</td>
<td>140</td>
</tr>
<tr>
<td>1 year</td>
<td>900</td>
<td>130</td>
</tr>
<tr>
<td>2 years</td>
<td>800</td>
<td>110</td>
</tr>
<tr>
<td>3 years</td>
<td>700</td>
<td>90</td>
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<tr>
<td>4 and 5 years</td>
<td>600</td>
<td>80</td>
</tr>
<tr>
<td>6 and 7 years</td>
<td>500</td>
<td>60</td>
</tr>
<tr>
<td>8 and 9 years</td>
<td>400</td>
<td>50</td>
</tr>
<tr>
<td>10–15 years</td>
<td>300</td>
<td>30</td>
</tr>
</tbody>
</table>

NTproBNP, N-terminal pro-brain natriuretic peptide.

Results

Figure 1 presents the Z-score curves of normal NTproBNP level according to age. NTproBNP concentration was highest between 1 and 11 months of age (approximately 140 pg/ml), infants <1 month of age had extremely high NTproBNP and equivalent degrees of freedom (ie, M:3, S:2, L:2). The distribution of the Z-score in KD patients was then compared with that of the control group (Figure 2).
Additionally, we established simple cut-offs every 100 pg/ml according to age for more convenient use at the bedside (Figure 4; Table 2). To each age bracket, the cut-offs for NTproBNP were 1,000 pg/ml for 1–11 months of age, 900 pg/ml for 1 year of age, 800 pg/ml for 2 years, 700 pg/ml for 3 year, 600 pg/ml for 4 and 5 years of age, 500 pg/ml for 6 and 7 years of age, 400 pg/ml for 8 and 9 years of age, and 300 pg/ml for 10–15 years of age. When we used these cut-offs, the specificity and sensitivity of NTproBNP for diagnosing acute KD was 98.0% and 43.6%, respectively (Table 1).

Figure 5 presents NTproBNP in each patient at different days of illness. Many patients had the highest level within 5 febrile days, but some patients had the highest level after 5 febrile day. In all KD patients (n=149), 65 patients exceeded the cut-off, and 49 patients (75.4%) had the highest level before i.v. injection of immunoglobulin (IVIG) treatment. There were 30 patients in the present study who fulfilled 4 of 6 criteria of Kawasaki, and were identified as having coronary aneurysm or dilatation on 2-D echocardiography. Among them, 15 patients exceeded the cut-off, and 11 patients (73.3%) had the highest level before IVIG treatment.

Discussion

BNP and NTproBNP are the most extensively studied and validated biomarkers for chronic heart failure in adults. These biomarkers provide information regarding elevated filling pressure, as well as chronic cardiac dysfunction and remodeling. BNP is synthesized in myocytes as proBNP, released in response to ventricular stress, and subsequently cleaved into the active peptide hormone (BNP) and inactive N-terminal peptide fragment (NTproBNP). BNP, which is degraded by endopeptidases, has a half-life of 5–10 min. NTproBNP is inactive, cleaved primarily by the kidneys, and has a half-life of 25–120 min.13

It has been shown that plasma BNP level markedly increases not only in patients with congestive heart failure, but also in those with acute myocardial infarction; the precise mechanism, however, is unclear. Kawamura et al hypothesized that 2 possibilities may account for the elevation in BNP level.14 One mechanism for this increase may be a result of local myocardial inflammation or areas of ischemia. Another mechanism may involve cytokines. It is known that tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-1β, and interferon-γ are present in the acute phase of KD. The released TNF-α induces vascular endothelial cells to express adhesive factors that prime neutrophils and monocytes. Furthermore, it also acts on endothelial cells and fibroblasts, induces various chemokines, facilitates migration of inflammatory cells to the inflammatory site, and increases production of cytokines.15 Thus, cytokines may encourage the secretion of BNP in the acute phase of KD.

Dahdah et al suggested that NTproBNP is a better marker of myocardial involvement in acute KD than BNP, particularly in cases of incomplete diagnostic criteria.16 This is because patients with incomplete diagnostic criteria for KD are less likely to be elevated BNP. Another advantage of NTproBNP, compared with BNP is its stability at room temperature. NTproBNP can be measured using only 0.1 ml of serum, enabling measurement in retrospect using archived serum.

The relationship between serum NTproBNP level and degree of KD severity is unclear. Delayed treatment, however, results in an increased incidence of aneurysms. Currently, KD is diagnosed based on clinical symptoms, but these are subjective. Biomarkers, such as NTproBNP, can be used to diagnose early-phase KD objectively. In the present study, almost 75% of KD patients had the highest NTproBNP level before treatment. This suggests that NTproBNP may be used as an adjunctive marker for acute-phase KD. Using this marker, we may be able to diagnose KD in the early phase and thus avoid delayed treatment. In other words, almost 25% of KD patients have the highest level after treatment, which may correlate somewhat with aneurysms often occurring after fever has subsided. Kaneko et al reported that NTproBNP is useful for the prediction of the risk of CAL in KD.7 And Maeno et al suggested that vascular endothelial growth factor (VEGF) was involved in the pathogenesis of KD, especially in the formation of CAL.17 When VEGF level was compared in patients with and without CAL, significantly higher VEGF level was observed in the subacute stage (10–20 days after onset) in patients with CAL,18 and in the acute stage (within 9 days after onset) in patients without CAL. There may be some relationship between the pathogenesis of CAL and the elevation of VEGF and NTproBNP after treatment. Further investigations are warranted to clarify this.

When we used the age-based cut-offs, the specificity of NTproBNP for diagnosing acute KD was 98.0%. Thus, NTproBNP appears to be a useful marker for diagnosing acute KD. Sensitivity, however, was not high, and KD cannot be excluded when serum NTproBNP is lower than the cut-off. Diagnosis should then be based on both clinical criteria and laboratory findings. Further investigations are warranted to identify novel markers that facilitate the diagnosis of KD. In particular, diagnostic tools are needed in the case of patients with incomplete KD. A limitation of the present study is that because of the limited number of patients with incomplete diagnostic criteria, we could not determine the usefulness of NTproBNP for incomplete KD (only 15 out of 30 patients with incomplete KD had <±2 Z-score).

Conclusions

We characterized the Z-score curve of NTproBNP level in children. Z-score >2.0 of NTproBNP or the identified cut-offs in children may be used to diagnose acute KD. There is also a possibility that NTproBNP can be used as the initial screening tool for cardiac disorders in children, for example, for initial diagnosis of cardiomyopathy and acute forms of myocarditis. Additionally, Z-scores or cut-offs of NTproBNP may be a useful follow-up tool for congenital heart disease and chronic heart disease.

Disclosures

Funding Source: No external funding was secured for this study. Financial Disclosure: The authors have no financial relationships relevant to this article to disclose. Conflict of Interest: The authors have no conflicts of interest to disclose. Clinical Trial Registration: None.

What’s Known on This Subject: Serum levels of NTproBNP are often elevated in patients suffering from acute KD. However, NTproBNP levels in normal infants and children are higher than those of adults.

What This Study Adds: We characterized the Z-score curve of NTproBNP levels in children. A Z-score >2.0 of NTproBNP or the determined cutoff values in children may be used to diagnose acute KD.

References


