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REVIEW

# NT-Pro-B-type Natriuretic Peptide in Infants and Children: Reference Values Based on Combined Data from Four Studies

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**Abstract** In cardiology, B-type natriuretic peptide and the amino terminal segment of its prohormone (NT-proBNP) are important biomarkers. The importance of these peptides as markers for heart disease in pediatric cardiology is reviewed. The peptide levels are dependent on age, assay, and possibly gender. The normal value range and upper limits for infants and children are needed. To determine reference values, data were combined from four studies that measured NT-proBNP levels in normal infants and children using the same electrochemiluminescence assay. The age intervals for the upper limits of normal were chosen for intervals in which no age-dependent change was observed. Statistical analysis was performed on log-transformed data. A total of 690 subjects (47% males) ages birth to 18 years were included in the review. The levels of NT-proBNP were highest in the first days of life, then showed a marked decline in the first week or weeks. The peptide levels continued to decline gradually with age ($r = 0.43$; $p < 0.001$). Male and female levels differed only for children ages 10 to 14 years. However, the upper limit of normal did not differ between the boys and girls in any age group. The findings lead to the conclusion that B-type natriuretic peptide (BNP) and NT-proBNP are important markers for heart disease in pediatric cardiology. The levels of NT-proBNP are highest in the first days of life and decrease drastically thereafter. A mild gradual decline occurs with age throughout childhood. Girls have somewhat higher levels of NT-proBNP during puberty.

**Keywords** Children · Heart disease · Infants · Natriuretic peptides · Reference values

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## The Natriuretic Peptides and the Heart

The natriuretic peptides are a family of structurally similar peptides. These peptides play an important role in the regulation of extracellular fluid volume and blood pressure. They induce natriuresis, diuresis, and vasodilation and specifically act to counter the effects of stress hormones such as those produced by the renin-angiotensin-aldosterone and adrenergic systems and endothelin [17]. The cardiac members of this peptide family are the atrial or A-type natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). Whereas ANP is secreted primarily from the cardiac atria in response to increased left and right atrial pressure and volume loads, BNP is secreted primarily from the ventricles in response to increased left and right ventricular pressure and volume loads.

The primary stimulus for natriuretic peptide release is myocyte stretch [7, 17]. The natriuretic peptides are synthesized as pre-prohormones, are cleaved to prohormones, and at release from the cells, are cleaved to a C-terminal biologically active peptide (BNP) and an N-terminal prohormone (NT-proBNP) that has no known biologic action. Both segments are found in the plasma.

Plasma levels of the natriuretic peptides are elevated in many cardiac diseases. Thus, they serve as markers for heart disease. Comparative studies have shown that BNP and its N-terminal prohormone fragment, NT-proBNP, are superior to ANP as markers [18]. The correlation between plasma levels of BNP and NT-proBNP in adults are shown to be good [3].

In the pediatric age group, head-to-head comparisons of BNP and NT-proBNP values in the same samples from both healthy children and those with congenital heart disease using different kits [12] also have shown good correlation. Both BNP and NT-proBNP can be measured on commercial laboratory platforms and are widely used [22].

Plasma levels of BNP and NT-proBNP are elevated in adult patients with a wide range of heart diseases including left ventricular systolic [7, 17] and diastolic dysfunction [16], ischemic heart disease, hypertrophic cardiomyopathy [21] and other cardiac diseases.

## The Natriuretic Peptides in Pediatric Cardiology

The results of studies addressing the natriuretic peptide level in children with heart disease are similar to those of studies with adults. In general, pediatric studies have found a correlation between the clinical heart failure score and BNP and NT-pro-BNP levels [24]. Elevated peptide levels were found in patients with heart failure from structural heart disease as well as in patients with dilated cardiomyopathy [34]. Infants with respiratory distress due to heart disease were found to have significantly higher plasma NT-proBNP levels than infants with respiratory distress due to lung disease or control children [6]. These findings suggest that peptide levels can differentiate between infants with respiratory distress due to heart disease and those with respiratory distress due to lung disease. For children with ventricular septal defect, plasma BNP levels correlated with the pulmonary-to-systemic flow ratio, the mean pulmonary artery pressure, and the pulmonary-to-systemic vascular resistance ratio [30]. Findings show that BNP identifies allograft disease in infants and children after cardiac transplantation [5]. Patients with acute Kawasaki disease were shown to have higher BNP levels than patients with acute viral illness or patients with Kawasaki disease in the convalescence phase [11, 31]

Other lesions for which BNP and NT-proBNP have known or potential value as markers showing the presence and severity of the disease include obstructive lesions [8], pure right heart disease or right ventricular dysfunction secondary to pulmonary hypertension [27], and complex single ventricle lesions after the Fontan operation [15].

Recent studies have shown that BNP and NT-proBNP levels can predict prognosis in various clinical situations. For children with dilated cardiomyopathy, elevated BNP levels were associated with future cardiac death, hospitalization, or listing for transplantation [25]. For pediatric patients admitted to the intensive care unit with decompensated heart failure due to various etiologies, high predischarge BNP levels were associated with adverse outcome within the following 60 days [32]. For children undergoing open heart surgery to manage congenital heart disease, BNP and NT-proBNP were shown to predict the postoperative course [2, 9, 10, 29].

## Normal Values in Infants and Children

A number of studies have reported on normal value ranges of the natriuretic peptides during infancy and childhood. Most studies show high levels of BNP and NT-proBNP immediately after birth [13, 20, 23, 35] and a decrease during the first weeks of life. A few studies show constant peptide levels after the neonatal period [20, 23, 33], whereas others report a tendency [19] or significant decrease in the peptide levels with age [26]. In contrast, Kunii et al. [14] found a nonsignificant tendency for BNP levels to increase with age. Regarding gender differences, Koch and Singer [13] found a sex-related difference in the second decade of life, with higher BNP concentrations in girls. In another study, NT-proBNP levels showed no gender- or age-related differences [19]. Different assays were found to provide different peptide values [1].Thus, the peptide levels are dependent on age, assay, and possibly gender in the pediatric age group. The normal value range and the upper limits of normal are essential to facilitate the use of these markers in pediatric cardiology. We present a summation of four studies [1, 23, 26, 28] that measured NT-proBNP levels in normal infants and children using the same assay. It is, to the best of our knowledge, the largest published data analysis to date.

## Reference Values of NT-proBNP in Normal Infants and Children

This report combines data from four studies that used the electrochemiluminescence immunoassay. The assay was performed with the Elecsys system 1010/2010 using the proBNP kit (Roche, Mannheim, Germany). Elecsys proBNP contains polyclonal antibodies that recognize epitopes located in the N-terminal part (1–76) of proBNP (1–108). The assay range is 5 to 35,000 pg/ml. The proBNP kit is unaffected by icterus (bilirubin <35 mg/dl), hemolysis (Hb <1.4 g/dl), or lipemia (TG 4000 mg/dl). No cross-reactivity (<0.001%) was observed with ANP, NT-pro-ANP, BNP, C-type natriuretic peptide (CNP), adrenomedullin, aldosterone, endothelin, renin, urodilatin or Arg-vasopressin, or angiotensin I, II, or III. Blood was collected via peripheral venous puncture and collected in tubes containing ethylenediaminetetraacetic acid (EDTA).

## Data Collection

The study included subjects who had blood taken for other reasons. Blood was collected from healthy newborns and from children and adolescents with uncomplicated diseases such as mild upper respiratory tract infections, motor and development disorders, social and behavioral problems, and neurologic disorders such as migraine. Blood also was taken from patients before elective surgery and from infants and children who had endocrinologic workup or routine infant screening.

Data from the four studies were evaluated together. A normality test (Kolmogorov–Smirnov) was performed on the original data and log-transformed data. A normal distribution was observed only for the log-transformed data. Statistical analysis was performed on the log data, and the results were converted back to the real values. Correlations were tested using Pearson's test. All age intervals were tested, and the intervals that showed no age-dependent change in peptide levels were determined. The 5th, 95th, and 97.5th percentiles were calculated for the chosen age intervals. Comparison of male and female values for all ages was performed using the unpaired Student's *t* test.

## Pediatric NT-proBNP Levels

Of the 690 subjects, ranging in age from birth to 18 years, 325 (47%) were boys. No data were available for the ages of 12 days and 1 month. The normality test (Kolmogorov–Smirnov) failed for NT-proBNP values (age, 2–18 years; $Z = 3.413$; $p < 0.001$), but passed for log-transformed values ($Z = 0.999$; $p = 0.271$; Fig. 1). The median and range of the NT-proBNP values as well as the upper and lower limits of normal (5th, 95th, and 97.5th percentiles) for the various age groups are shown in Table 1. The age intervals of 1 to 2 years showed declining levels. However, for practical reasons, shorter periods were not used.

The NT-proBNP levels were very high after birth, then decreased drastically in the first days. The peptide levels continued to decline gradually with age, showing a significant decrease between the ages of 1 month and 18 years ($r = 0.43$; $p < 0.001$; Fig. 2). The levels differed between the boys and girls only for the group 10 and 14 years of age (medians: boys, 38 pg/ml; girls, 56.5 pg/ml; $p = 0.002$). However, the upper limit of normal for the boys and girls was not different in any age group (Fig. 3). The 95th percentile for normal NT-proBNP levels by age are as follows: 0 to 2 days (11,987 pg/ml), 3 to 11 days (5,918 pg/ml), 1 month to 1 year (646 pg/ml), 1 to 2 years (413 pg/ml), 2 to 6 years (289 pg/ml), 6 to 14 years (157 pg/ml), and 14 to 18 years (158 pg/ml).

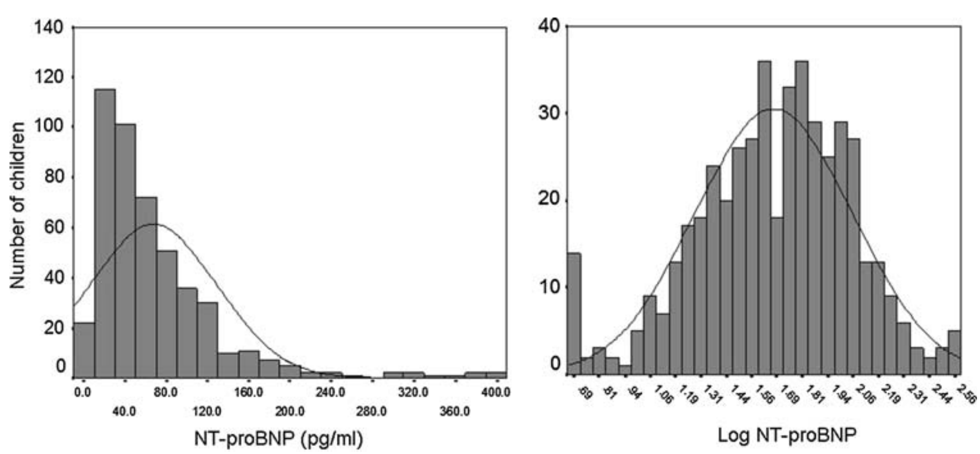


**Fig. 1** Histogram showing N-terminal prohormone (NT-proBNP) values of 475 normal children and adolescences ages 2 to 18 years. Left: Histogram of the original data showing a non-Gaussian distribution with left truncation of the Gaussian bell. The normality test (Kolmogorov–Smirnov) failed ($Z = 3.413$; $p < 0.001$). Right: Log transformation of the data, resulting in a normal distribution ($Z = 0.999$; $p = 0.271$)

**Table 1** NT-proBNP levels (pg/ml) of normal infants, children and adolescence from birth to 18 years of age

| Age interval | *n* | Median (pg/ml) | Range (pg/ml) | 5%tile | 95%tile | 97.5%tile |
|---|---|---|---|---|---|---|
| 0–2 d | 43 | 3,183 | 260–13,224 | 321 | 11,987 | 13,222 |
| 3–11 d[a] | 84 | 2,210 | 28–7,250 | 263 | 5,918 | 6,502 |
| >1 mo to ≤1 yr | 50 | 141 | 5–1,121 | 37 | 646 | 1,000 |
| >1 to ≤2 yr[b] | 38 | 129 | 31–675 | 39 | 413 | 675 |
| >2 to ≤6 yr | 81 | 70 | 5–391 | 23 | 289 | 327 |
| >6 to ≤14 yr | 278 | 52 | 5–391 | 10 | 157 | 242 |
| >14 to ≤18 yr | 116 | 34 | 5–363 | 6 | 158 | 207 |

[a] No data for patients 12 to 30 days of age

[b] A significant decrease with age in this interval

## Comments

Currently, both BNP and NT-proBNP are used in clinical cardiology practice. Normal values are essential to the interpretation of results for an individual patient. Because values are assay dependent, every kit establishes its own limits of normal. The assay difference was demonstrated by Albers et al. [1], who compared the Roche NT-proBNP assay with the Biomedica NT-proBNP assay. These authors found the mean difference between the assays to be 1649.7 ng/l.

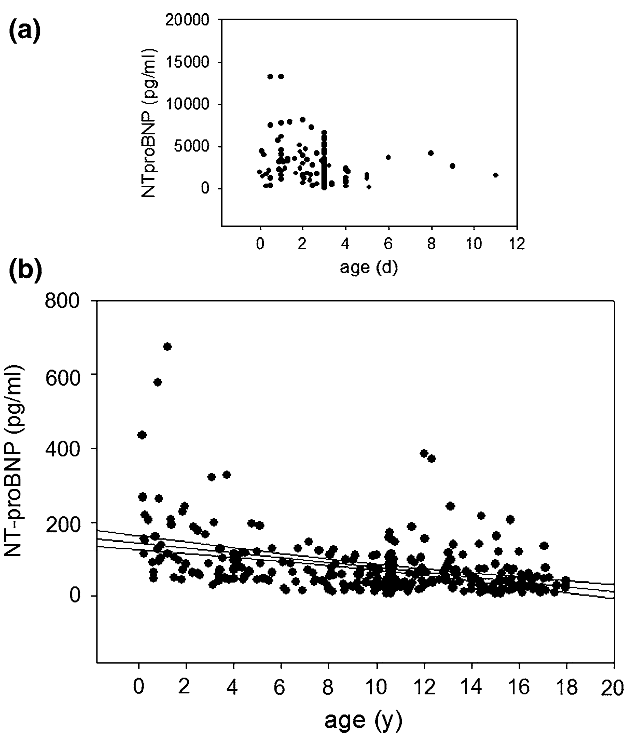


**Fig. 2** (**a**) Scatter plot of NT-proBNP levels in normal newborns from birth to 11 days. (**b**) Scattered plot showing the N-terminal prohormone (NT-proBNP) levels of normal infants, children, and adolescents ages 1 month to 18 years

The current analysis aimed to establish reference values and upper limits for infants and children for the Roche NT-proBNP kit. For this purpose, four published studies that measured the peptide levels in normal pediatric populations were combined. Not all the subjects were completely healthy. Some of the patients had mild upper respiratory tract infections or other noncardiac conditions. Because complete cardiac evaluation was not performed, it is theoretically possible that a small minority of the subjects had altered NT-proBNP levels due to their conditions. It is, however, extremely unlikely that a child with no history or physical findings of heart disease will have a significant heart disease.

The results show, as suggested in the past, that peptide levels do not follow normal Gaussian distribution, but that log-transformation of the values does. Log-normal distribution often is observed for variables that result from multiplying several different sources of variation. This

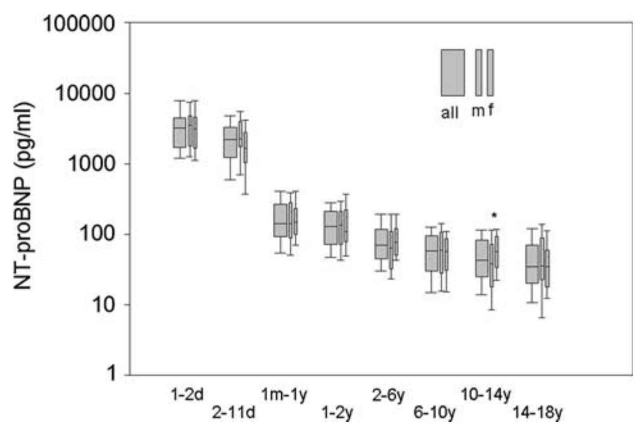


**Fig. 3** Box-plot showing, on a logarithmic scale, N-terminal prohormone (NT-proBNP) levels for normal infants and children in the different age intervals. Box lines represent median, 5th, 25th, 75th, and 95th percentiles. For each age interval, the left (*thick*) bar represents the whole group, the middle (*thin*) bar represents males, and the right (*thin*) bar represents females. *$p < 0.05$ between males and females

often happens with variables that are part of metabolic pathways in which the rate that a reaction can take place depends on concentrations of other compounds [4]. The log normality of NT-proBNP suggests such biologic properties.

Controversy exists about whether NT-proBNP levels are stable or decline beyond the neonatal period. A statistically significant decline in the levels beyond the neonatal period was found by Albers et al. [1], Rauh and Koch [26], and Schwachtgen et al. [28], but not by Nir et al. [23]. The current study supports the view that gradual decline occurs during childhood and approaches published adult levels in adolescence.

Another unclear issue involves the possible differences between boys and girls. The findings of this study support higher NT-proBNP levels in pubertal girls. This was reported by Schwachtgen et al. [28], but not by Albers et al. [1], Nir et al. [23], or Rauh and Koch [26]. A very important aspect of this report is the establishment of upper limits of normal based on the largest number of subjects reported to date. If the 95th percentile is taken as the upper limit of normal, it seems safe to declare levels above the following figures to be elevated: first 2 days of life (>12,000 pg/ml), days 3 to 11 (>6,000 pg/ml), 1 month to 1 year (>650 pg/ml), 1 to 2 years (>400 pg/ml), 2 to 6 years (>300 pg/ml), and 6 to 18 years (>160 pg/ml).

## Conclusions

In pediatric cardiology, BNP and NT-proBNP are important markers for heart disease. The NT-proBNP levels in healthy subjects are very high during the first days of life but decrease drastically thereafter. There is a mild gradual decline with age throughout childhood. Girls have somewhat higher NT-proBNP levels during puberty.

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