

# Reference intervals for brain natriuretic peptide in healthy newborns and infants measured with an automated immunoassay platform

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## Abstract

**Background:** In order to assess the reference intervals for B-type natriuretic hormone (BNP) in the first days of life, we measured peptide concentrations using the fully automated Access platform.

**Methods:** Plasma BNP was measured in 188 apparently healthy newborns and infants throughout the first month of extra-uterine life, as well as in 245 healthy infants ranging from 1 month to 12 years of age.

**Results:** BNP showed the highest concentrations in the first 2 days of life, with a progressive decline afterwards. Moreover, BNP values in the first week of life were significantly higher ( $p < 0.0001$ ) than values observed in the next periods. As a result, a significant negative correlation was found between BNP and age values when considering all 433 samples ( $\rho = -0.816$ ,  $p < 0.0001$  by the Spearman rank correlation test). There was no significant difference between BNP values found in males and females.

**Conclusions:** According to this data, our study indicates that at least two reference intervals should be used for newborns and infants. The first, with higher BNP values for neonates in the first week of extra-uterine life, and the other, with lower BNP values for infants aged 2 weeks to 12 years.

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**Keywords:** B-type natriuretic hormone; infants; natriuretic peptides; newborns; reference values.

## Introduction

The measurement of circulating B-type natriuretic hormone (BNP) and its related peptides is now considered a useful

marker of myocardial function. In particular, as recently recommended by the Task Force of the European Society of Cardiology (1), measurement of BNP assay was included in the first step of the algorithm for the diagnosis of heart failure, along with an ECG and a radiogram of the thorax. Some meta-analyses (2–4) have confirmed that BNP has a high degree of diagnostic accuracy and clinical relevance in both acute and chronic heart failure in adults.

As recently reviewed, several studies reported the clinical usefulness of BNP as a marker for heart disease in pediatric patients (5–7). However, BNP values may be affected by some physiological conditions (2, 8), as well as by some analytical characteristics of the immunoassay methods used for its measurement (9–11). In particular, it is well known that BNP values are age and gender dependent in adult subjects (2, 8). Moreover, commercial assays are affected differently by the presence in plasma samples of several peptides derived from degradation of intact prohormone and BNP (9, 10). Therefore, BNP methods are affected by large systematic differences (up to three-fold between commercially available methods) (11).

There is little data on the reference intervals for BNP, and its related peptides, such as the N-terminal fragment of proBNP (NT-proBNP) in infancy (5, 6, 12, 13). Very recently, Nir et al. (6) measured NT-proBNP concentrations with an electrochemiluminescent assay method in 690 healthy subjects (47% males) ranging from birth to 18 years of age, including 127 newborns in the first week of life (43 in the first 2 days of life). The concentrations of NT-proBNP were highest in the first days of life after which they showed a marked decline. Concentrations in males and females differed only for children aged 10–14 years. Reference intervals for BNP in newborns and infants have not been reported in any large studies (5). At present, there are data from only two relatively small studies (12, 13). In order to determine reference intervals for BNP in the first days of life, we measured the peptide concentrations using a fully automated immunoassay platform (Access Immunoassay Systems, Beckman Coulter, Inc., Fullerton, CA, USA).

## Materials and methods

### Plasma samples

Plasma BNP was measured in the residual EDTA plasma from blood samples (usually 0.2–0.4 mL) collected from a superficial venous vessel on the heel of 188 apparently healthy newborns throughout the first week of extra-uterine life. In addition, we collected plasma samples from another group of 245 healthy infants with ages rang-

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ing from 1 week to 12 years. Fifty-seven percent of neonates and infants enrolled in the study were males. Blood was taken from healthy newborns and infants who had undergone routine screening for genetic disorders (for newborns) or during screening for endocrinological disorder (for infants).

All newborns were delivered at term (between 35 and 42 weeks of gestation) and had a body weight at birth ranging from 2.5 to 4.1 kg with an Apgar score  $\geq 8$ . Twenty-five percent of deliveries were by elective cesarean section. In infants, clinical examination excluded the presence of acute illness (i.e., for infections or electrolyte imbalance) and laboratory test results were within the reference limits.

In order to minimize degradation of plasma BNP (2, 8), blood samples were placed in disposable polypropylene tubes containing EDTA (1 mg/mL of plasma) immediately following collection. Plasma was obtained rapidly by centrifugation for 15 min at 4°C, and BNP measurements were performed as soon as possible (no more than 4 h following collection of blood).

Informed consent was given from the parents of newborns and infants enrolled in this study.

### BNP assay

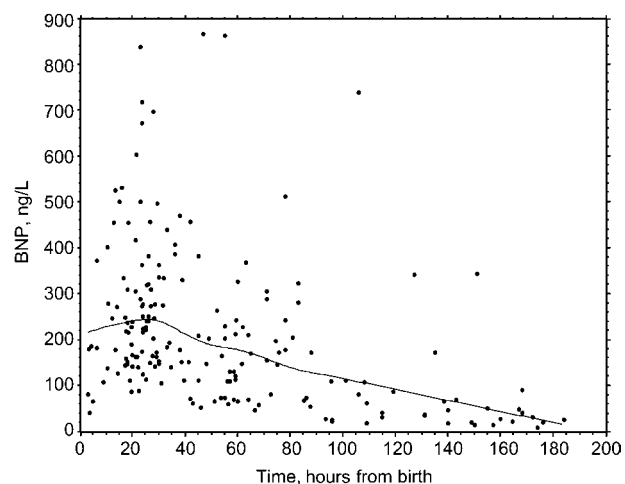
Plasma BNP was measured using the fully automated Access platform (Triage BNP reagents, Access Immunoassay Systems, REF 98200; Beckman Coulter, Inc., Fullerton, CA, USA). The analytical characteristics and performance of the Access immunoassay method used in this study for measurement of BNP were previously evaluated in our laboratory (14). In particular, the analytical detection limit of the Access method, tested by repeatedly measuring ( $n=20$ ) the calibrator with a BNP concentration of 0 ng/L in three different runs, was 0.30 ng/L (14). Within-run and total imprecision of the Access method was tested following the CLSI EP5-A guidelines. We performed repeated measurements of two plasma samples over 20 working days, in duplicate, with BNP concentrations of 52.6 ng/L and 1095 ng/L; within-run imprecision for the two samples was 3.39% and 1.56%, and total imprecision was 8.44% and 5.92%, respectively (14).

### Statistical analysis

Standard statistical analysis was performed using a Macintosh PowerPC G5 with the Stat-View 5.0.1 program (1992–98, SAS Institute Inc., Cary, NC, USA). Because circulating BNP concentrations are not normally distributed in healthy subjects, both the original and logarithmic transformation of data was used for statistical analysis. The analysis of the trend of BNP values in the first week of life, reported in Figure 1, was assessed by smooth spline analysis with a LOWESS (Locally Weighted Scatterplot Smoother) of 66% (15). For the same reason, non-parametric tests were used, and the median and percentile values were reported.

### Results

BNP concentrations measured in the 188 newborns during the first week of life are reported in Figure 1 and Table 1. BNP showed the highest concentrations during the first 2 days of life, with a progressive decline thereafter. Indeed, both BNP values measured on the first day and second day were significantly higher than in the following 49–96 h ( $p<0.01$  by Scheffè post hoc ANOVA test using log-transformed original values) and 96–184 h ( $p<0.0001$ ) of life,



**Figure 1** Trend and distribution of BNP values obtained in 188 healthy newborns throughout the first 184 h of life. The trend is indicated by a continuous line, assessed by smooth spline analysis using a LOWESS of 66% (15).

respectively (Table 1). The 99th and 97.5th percentiles of the distribution of BNP values during the first week of life were 852.5 ng/L and 713.8 ng/L, respectively.

BNP values in the 245 infants, grouped according to three time periods from the second week to 12 years of life, are also reported in Table 1. BNP values from the 8th day to 30th day were significantly higher than those found in the other two periods ( $p=0.0022$  compared to the 31 days–12 months group, and  $p<0.0001$  compared to the 1–12 years groups using Scheffè post hoc ANOVA test with log-transformed original values, respectively). The 99th and the 97.5th percentiles of BNP concentrations in these three groups of infants, taken as a whole, were 39.6 ng/L and 37.0 ng/L, respectively.

The data reported in Figure 1 and Table 1 confirmed that BNP values in the first week of life were significantly higher than the values observed in the following time periods ( $p<0.001$  by Scheffè post hoc ANOVA test using log-transformed original values). As a result, a significant negative correlation was found between BNP and age when all 433 samples were evaluated together ( $\rho=-0.816$ ,  $p<0.0001$  by the Spearman rank correlation test). There was no significant difference between BNP concentrations in males and females [males: mean 101.6 ng/L, standard deviation (SD) 140.2 ng/L, median 28.0 ng/L,  $n=246$ ; females: mean 108.0 ng/L, SD 158.1 ng/L, median 31.0 ng/L,  $n=187$ ].

### Discussion

The current study reports the reference intervals for BNP, measured using an automated immunoassay, in a population of 433 healthy newborns and infants from birth to 12 years of age. The present results confirm data previously described by our laboratory in a preliminary report, where BNP values from 127 newborns during the first week of life were considered (13). Our data on the circulating concentrations of

**Table 1** Distribution BNP concentrations (ng/L), grouped according to time periods from birth to 12 years of age, measured in 433 healthy newborns and infants.

Groups (time periods from birth)	Number of individuals	25th percentile	Median	75th percentile	90th percentile	Range	p-Value
0–24 h	57	151.3	224.0	341.5	521.0	41–837	<0.0001
25–48 h	49	152.8	242.0	343.5	457.0	53–866	<0.0001
49–96 h	50	74.0	152.0	229.0	315.0	23–862	<0.0001
97–192 h	32	25.0	45.0	89.5	224.4	10–739	<0.0001
8–30 days	34	17.0	27.0	45.0	55.2	9–63	0.1144
31 days–12 months	69	11.0	19.0	28.0	36.0	1–53	0.7303
1–12 years	142	10.0	14.5	20.0	28.3	1–46	

Range, minimum and maximum values; p-value concerning comparisons with the “1–12 years” group by Scheffè post hoc test after ANOVA using log-transformation of original BNP values.

BNP, which is a biologically active hormone, are in agreement with those reported by Nir et al. (6) for NT-proBNP, which is an inactive peptide with a longer half-life. Indeed, plasma concentrations of both BNP and NT-proBNP are highest during the first 2 days of life, with a progressive decrease over the following days and weeks. Soldin et al. (12) reported slightly higher values when BNP (97.5th percentile = 1585 ng/L) was measured in 50 EDTA whole blood samples using a point-of-care testing (POCT) method over the first month of life.

As expected, we found no difference in BNP values between male and female infants. Our data confirm the hypothesis that gender-dependent BNP values in adult subjects are due to the action of steroid sex hormones (10, 16, 17), and these effects become evident only after sexual maturation.

Increased circulating concentrations of cardiac natriuretic hormones, including both atrial natriuretic peptide (ANP) and BNP and their related peptides, in the first days of extra-uterine life have been described previously (5, 6, 12, 13, 18). It is well known that the transition from fetal to neonatal circulation is accompanied by an increase in pulmonary blood flow as a result of lung expansion and an increase in systemic vascular resistance. The perinatal circulatory changes lead to an increase in ventricular volume and pressure load, and this may stimulate the synthesis and secretion of cardiac natriuretic hormones by atrial and ventricular cardiomyocytes. As suggested previously (18), the increased concentrations of natriuretic hormones may act to alleviate the increased ventricular load after birth and may also support heart function with a decreased preload in the first days of life.

Our study has some limitations. Despite the growing number of biomarkers, efforts to recognize and treat high cardiovascular risk children have been largely unsuccessful. This is due primarily to the lack of appropriate reference intervals to aid clinicians in the interpretation of measured values (17). Many of the reference intervals that have been published to date cover limited pediatric age ranges, involve hospitalized children and do not analyze each gender separately (17). In particular, sample size is an important factor to take into account for the estimation of population reference intervals. International guidelines recommend a minimum of 120 reference individuals per group for appropriate statistical deter-

mination of reference limits (19). Owing to the obvious limitations in obtaining plasma samples in healthy newborns, there is limited data concerning reference intervals for BNP, based on large (more than 100 individuals) population studies (5, 6).

From a clinical point of view, the most important information from our study is that BNP values are highest in the first 4 days of life (Figure 1), fall rapidly during the first week of extra-uterine life, and then fall more slowly throughout the first month of life (Table 1). Finally, BNP concentrations seem to remain steady, without any significantly change, from 31 days to 12 years of age (Table 1). According to this data, an upper limit of the reference interval of ~40 ng/L, close to the 99th percentile of BNP concentrations, may be recommended in infants of 1 month (or more) of age. Due to large variations of BNP values, the diagnosis of cardiac disease may be more difficult to make in newborns throughout the first week of extra-uterine life (Figure 1). Indeed, a higher upper limit of the reference interval should be used (e.g., 850 ng/L, corresponding to 99th percentile of BNP distribution values in the first week of life). Of course, more accurate decision values should be assessed using clinical studies, including pediatric patients with different cardiac diseases, where the diagnostic accuracy of BNP can be calculated using appropriate statistical analysis.

Another limitation of our study is that BNP values are strongly method dependent (2, 8, 11). Therefore, the reference intervals obtained in this study with the fully automated Access platform cannot be adopted directly by other laboratories that use different platforms or immunoassay systems. As a result, clinicians should take into account the method used for the measurement of BNP reported in the literature, or by other laboratories, when they compare these values to those found in their own laboratory.

In conclusion, our study confirms that plasma BNP concentrations are highest in the first 4 days of life, with a rapid decrease in the next days and weeks. According to this data, our study indicates that at least two upper limits of the reference limit for BNP should be considered for newborns and infants. The first with higher BNP values should be used for neonates during the first week of extra-uterine life, while the other with lower BNP values should be used for infants and children from 2 weeks to 12 years of age.

## Conflict of interest statement

**Authors' conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article.

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