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Pediatric brain natriuretic peptide and N-terminal pro-brain natriuretic peptide reference intervals

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Abstract

Background—Brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) have been shown to be useful biomarkers for the diagnosis of heart failure. Pediatric reference intervals for these analytes have been reported in part. Previous studies lack large numbers in each group, have not covered all age ranges and have not compared results for BNP with NT-proBNP in simultaneously drawn samples.

Methods—We measured BNP in whole blood using the Biosite Triage point-of-care method and plasma NT-proBNP using the Dade RxL Dimension. We assessed between and within-day precision of both methods and after removing outliers employed the Hoffmann approach to calculate pediatric reference intervals over the age range of 0–21 y. We also compared the 2 methods on simultaneously drawn samples.

Results—Reference intervals revealed approximately 20-fold higher 97.5th percentiles for neonates than for children >3 y of age. 97.5th percentiles decreased significantly over the first 3 years of life. As shown by others, the CVs for the automated Dade RxL platform were somewhat lower than those for the POCT method. BNP and NT-proBNP correlated well in simultaneously drawn samples (r=0.947).

Discussion—Reference intervals for BNP and NT-proBNP are far higher in neonates and infants than in children older than three years of age. The reasons for this are unknown but resemble the elevated CK-MBs and troponins also found in neonates, although the 97.5th percentiles for these latter 2 cardiac markers decrease more rapidly to values found in older children by 6 months of age.

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Keywords

BNP; NT-proBNP; Pediatric reference intervals

1. Introduction

Congestive heart failure (CHF) is the fourth leading cause of adult hospitalizations in the U.S. [1,2]. CHF is almost always a chronic, long-term condition, although it can sometimes develop suddenly [3]. Failure of both sides of the heart can affect most areas of the body. CHF is traditionally diagnosed by a history and physical examination. Supplementary diagnostic testing includes chest radiography, echocardiography, right-sided heart catheterization and the 6-min walk test. Because a patient's sudden onset of shortness of breath may be due to any of a variety of causes, clinicians need an inexpensive, simple, rapid and objective test that can be conducted at the point of care to aid diagnosis.

There are 3 major natriuretic peptides (NPs): atrial natriuretic peptide (ANP) synthesized in the atria, brain natriuretic peptide (BNP) synthesized in the ventricles, and C-type natriuretic peptide synthesized in the brain. BNP and NT-proBNP have been studied as biomarkers for the diagnosis, evaluation and management of heart failure. BNP, a cardiac hormone with diuretic, natriuretic and vasorelaxant properties, is secreted by the heart in response to ventricular expansion or pressure overload. The pro form (ProBNP) is cleaved, releasing the 32 amino acid active BNP and an N-terminal piece of 76 amino acids designated NT-proBNP. The concentrations of BNP and NT-proBNP are dependent on both their synthesis and clearance. Both BNP and NT-proBNP are markers of ventricular distension and overload and can serve as early diagnostic tests [4].

Plasma BNP concentrations correlate with elevated end-diastolic pressure, which closely parallels dyspnea in heart failure [4]. This suggests that BNP is uniquely suited to provide accurate profiling in heart failure, increasing as heart failure progresses. A normal BNP or NT-BNP level has a high negative predictive value for heart failure. Ruling out this diagnosis allows the clinician to spare the patient additional invasive and uncomfortable testing (e.g., echocardiogram, right heart catheterization) and to focus on other possible causes of shortness of breath. In patients with elevated BNP or NT-proBNP levels, the degree of elevation correlates with the severity of heart failure as measured by the New York Heart Association classification system [4]. BNP is elevated in patients who have right heart failure, but the degree of elevation is not as great as that associated with left ventricular dysfunction. Research is also being conducted to evaluate the utility of BNP and NT-proBNP for other purposes, including screening for left ventricular (LV) dysfunction and risk stratification in acute ischemic syndromes. Renal function has also been shown to influence the optimal cut-off points of NT-proBNP and BNP for the diagnosis of cardiac-related dyspnoea [5]. Adult reference intervals are available in the literature [6,7] and recently Hammerer-Lercher et al. and Bekker et al. [8,9] demonstrated that NT-proBNP concentrations are markedly higher in the umbilical cord blood of term newborns than in their mothers. Data evaluating BNP and NT-proBNP in children have been reported by Yoshibayashi et al. (BNP, n =58), Mir et al. (NT-proBNP, n =109) and Rauh et al. (NT-proBNP, n =91) [10–13]. All these studies have been performed on very small numbers of children at any one age group. In contrast, we have determined the reference intervals for BNP (n =808) and NT-proBNP (n =1207) with significant numbers for each sex and at each age group.

We measured BNP by the Biosite method (Biosite Inc., San Diego, CA) and NT-proBNP using the Dade RxL Dimension (Dade Behring Inc., Newark, DE). After removing outliers

(14) we have employed the Hoffmann approach [15] to obtain the pediatric reference intervals and have plotted the log of the value vs. percent cumulative frequency. A straight line was obtained which deviated from linearity at both ends. This straight line can be extended to provide the 2.5th and 97.5th percentiles for each sex and age range studied [14,15].This approach is well accepted and has been employed by our group for many years [14 –25]. Pediatric reference intervals for BNP and NT-proBNP have not previously been available in the literature.

2. Methods

Patient samples were obtained from left-over specimens and were from an approximate 50/50 mix of inpatient and outpatient specimens. All data except sex, analyte concentration and age were immediately deleted to assure compliance with hospital confidentiality standards. For the reference interval studies, EDTA whole blood was used for the BNP quantification using the Triage Biosite point-of-care method performed according to the manufacturer's standards. Here all assays were performed within 2 h of blood collection. Reference intervals for NT-proBNP were performed on plasma samples some of which were stored in the freezer (−20 °C) and measured on the Dade RxL Dimension analyzer within 2 days of blood collection. In another study, 160 patients had EDTA samples drawn simultaneously with plasma samples thereby allowing comparison between the methods. In this case all analyses were completed within 2 h of blood collection. Precision data were obtained at 3 concentration levels using BioRad controls.

3. Results

Fig. 1 shows the comparison of the Biosite BNP results with simultaneously drawn plasma samples for measurement of NT-proBNP using the Dade RxL Dimension. Table 1 provides the between and within-day imprecision of the BNP method. Table 2 gives the between and within-day imprecision of NT-proBNP assay. Table 3 shows the 97.5th percentiles for males and females over the age range 0 –21 y for BNP (pg/ml). Table 4 gives the 97.5th percentiles for males and females over the age range 0 –21 y for NT-proBNP (pg/ml).

4. Discussion

This study allowed us to compare one of the main point-of-care approaches (Biosite, Triage BNP) for the assessment of potential cardiac failure with one of the most commonly used laboratory platforms (Dade RxL Dimension, NT-proBNP). The correlation between simultaneously drawn whole blood BNP and plasma NT-proBNP is impressive (r=0.947, Fig. 1). Clearly, both systems have acceptable precision (Tables 1 and 2) and, not surprisingly, the CVs for the automated Dade RxL Dimension are lower than the manual and somewhat more subjectively read Biosite POCT approach, which supports the findings of Yeo et al. [6]. The better stability of NT-proBNP over the more labile BNP needs also to be taken into account [6]. These NT-proBNP advantages have to be weighed against the advantage of being able to run the test in the Emergency Room vs. the Main Laboratory with the former providing a somewhat faster turnaround-time. ProBNP is cleaved to give both BNP and NT-proBNP and our studies reflected in Fig. 1 reveal that this splitting resulted in a high correlation between BNP and NT-proBNP levels. NT-proBNP gives results almost 5-fold greater than BNP (slope in Fig. 1 is 4.87). The significant age related effect (20-fold for NT-proBNP and 18-fold for BNP) on the 97.5th percentiles shown in Tables 3 and 4 are similar for both BNP and NT-proBNP. While previous studies had inferred higher concentrations in the newborn period and infants, the n's used were very small for any sex or age group and consequently could not be used to reliably identify reference intervals [8 –13].We should emphasize that the 0–31 day group is comprised predominantly of premature infants ranging from 25 weeks gestational age to full term infants. It should also be noted

that similar age related effects were found for the cardiac markers CPK-MB and troponin [23,25]. Reasons for this are unknown but resemble the increased CK-MBs and troponins also found in neonates although the 97.5th percentiles for these latter cardiac markers decrease more rapidly to values found in older children by around 6 months of age.

This data is supported by the work of Hammerer-Lercher et al. and Bakker et al. [8,9] who showed NT-proBNP to be significantly higher in plasma from cord blood than in maternal plasma. *N* values in previous studies have been far too small to reliably define reference intervals for males and females at each age group [8–13]. Our data differs from that reported by Rauh et al. [13] and Koch et al. [26]. While both these papers show higher values for BNP and/or NT-proBNP in neonates and agree with our findings in this regard, their values appear to decline fairly rapidly with increasing age. The high levels in the neonate have been conjectured to be possibly due to physiological water loss that occurs in the first week of life [27,28]. Another possible reason is the possibility of peptide clearance by the placenta which ends at birth [27]. A close examination of their [13,26] data reveals that the number of male and female children studied at any one age group are very small with numbers of 1 to 6. This would question the reliability of their reference intervals. In comparison our number of subjects varied from 26 to 91 for BNP and from 23 to 112 for NT-proBNP. In the Hoffmann approach used in this study (and in almost all our previous studies) the top and bottom 20% of the data were automatically deleted. Heart anomalies (congenital heart disease, acquired heart disease such as myocarditis, cardiomyopathy, rheumatic fever, infective endocarditis, Kawaski disease) in pediatrics are found in <2–3% of the pediatric population [27,28] and these data points would have been automatically excluded from our population studied. We plotted the log of the value (BNP or NT-proBNP) vs. the percent cumulative frequency for the central 60% of the data. This resulted in a straight line drawn to the mid-60th percentile of the data. Extension of this line allowed us to calculate the 97.5th percentiles. As indicated approximately 50% of the samples were obtained from outpatients and 50% from inpatients. In our pediatric population only a very small percentage of patients (perhaps 2%) had cardiovascular disease and therefore the samples used were largely from children with normal cardiac function. The 2% abnormal results would clearly have been removed through elimination of the top 20% of the data.

We believe the age related 97.5th percentile data for these 2 important markers published in this manuscript should be helpful to clinicians attempting to assess cardiac failure and left ventricular dysfunction in children.

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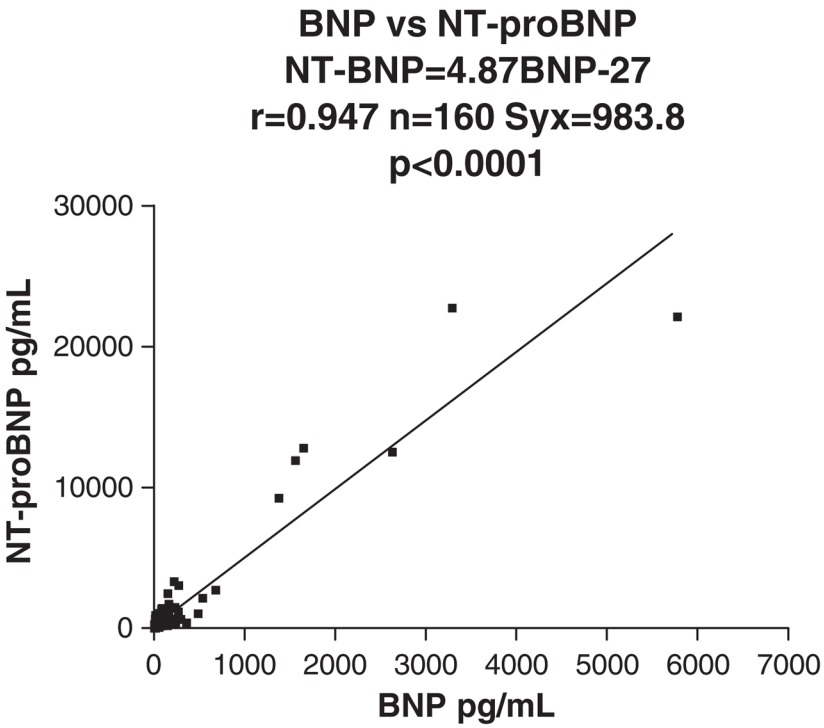

Fig. 1.
Biosite BNP measurements compared with simultaneously drawn plasma samples for the measurement of NT-proBNP using the Dade RxL Dimension.

Table 1

Between and within-day imprecision of the BNP method

	Level 1	Level 2	Level 3
Within-day precision			
CV	9.5%	8.9%	8.5%
SD	21.1	87.7	256
N	10	10	10
Mean pg/ml	222.2	987.1	3012.3
Between-day precision			
CV	5.5%	14.7%	13.8%
SD	11.0	122.9	386.4
N	10	10	10
Mean pg/ml	201.0	838.2	2807.8

Table 2

Between and within-day imprecision of NT-proBNP assay

	Level 1	Level 2	Level 3
Within-day precision			
CV	5.3%	3.1%	3.2%
SD	11.6	24.6	305.1
N	11	11	11
Mean pg/ml	218.2	792.8	9588
Between-day precision (using a different lot # from above)			
CV	5.8%	5.8%	3.9%
SD	20.0	73.1	491.9
N	10	10	10
Mean pg/ml	342.5	1270	12771

Table 3

97.5th percentiles for males and females over the age range 0 –21 years for BNP (pg/ml)

Age	Sex	97.5th percentile	*n*
0–<31 days	M and F	1585	50
31–<90 days	M and F	1259	38
3–<6 months	M and F	759	26
6 months–<1 y	M and F	263	55
1–<3 y	M	173	60
1–<3 y	F	158	51
3–<10 y	M	132	89
3–<10 y	F	120	72
10–<15 y	M	120	91
10–<15 y	F	115	51
15–<18 y	M	100	63
15–<18 y	F	107	66
18–21 y	M	110	50
18–21 y	F	87	46

Table 4

97.5th percentiles for males and females over the age range 0 – 21 y for NTproBNP (pg/ml)

Age	Sex	97.5th percentile	*n*
0–<31 days	M	28,184	46
0–<31 days	F	35,481	53
31–<90 days	M	19,953	49
31–<90 days	F	15,135	45
3–<6 months	M	15,849	49
3–<6 months	F	14,125	23
6 months–<1 y	M	11,220	40
6 months–<1 y	F	10,000	50
1–<3 y	M	5012	105
1–<3 y	F	2512	59
3–<10 y	M	1259	108
3–<10 y	F	1324	90
10–<15 y	M	1585	112
10–<15 y	F	1413	87
15–<18 y	M	1584	76
15–<18 y	F	1318	103
18–21 y	M	1600	61
18–21 y	F	1400	51