Amino Terminal pro B-Type Natriuretic Peptide Levels Are Elevated in the Cord Blood of Neonates With Congenital Heart Defect

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ABSTRACT: This prospective study investigates whether amino terminal proB-type natriuretic peptide (NT-proBNP) levels are elevated in neonates with congenital heart defect (CHD). NT-proBNP levels in the umbilical cord blood of 60 neonates with prenatally diagnosed CHD, in the cord blood of 200 control subjects, and in the plasma of the respective mothers were analyzed using an automated enzyme immunoassay. NT-proBNP concentrations in the cord blood of the CHD group were significantly elevated compared with controls [median (range): 158 pg/mL (378–3631 pg/mL) and 626 pg/mL (153–2518 pg/mL); p value <0.001]. The NT-proBNP concentrations of the newborns and their mothers did correlate neither in the CHD nor in the control group. In 54 patients with CHD, NT-proBNP levels were measured on the median 5th day of life. They showed a significant increase (median 1665 pg/mL and 19222 pg/mL; p < 0.001). NT-proBNP levels in the cord blood of neonates with CHD are significantly elevated and show a marked increase in the first week of life. Furthermore, this study confirms previously published reference intervals of NT-proBNP in the cord blood of healthy neonates as well as the finding that there is no exchange of NT-proBNP across the placenta. (*Pediatr Res* 66: 466–469, 2009)

Brain-type natriuretic peptide (BNP) is a natriuretic hormone secreted from the cardiac ventricular myocytes in response to an increase in ventricular wall stretch and filling pressures (1). Furthermore, hypoxia has been shown to be a direct stimulus for secretion of BNP in human cardiac myocytes (2). On secretion from the cardiac myocyte, pro-BNP, the inactive precursor, is cleaved into the biologically active BNP and the amino terminal fragment (NT-proBNP). Several studies have shown an excellent correlation between BNP and NT-proBNP plasma levels of adults, children, and in umbilical cord blood (3–5).

Concentrations of BNP and its biologically inactive fragment NT-proBNP are of high relevance in the diagnosis and treatment management of congestive heart failure in adults and children (3,6–11). Both peptides are useful markers in a variety of cardiac diseases in children and adolescents (11–18). It is well imaginable that also premature and newborn patients could benefit from the use of natriuretic peptide levels for diagnosis and treatment guidance of cardiac diseases.

However, data on the usefulness of natriuretic peptides for diagnosis and management of congenital heart disease in this age group are limited. Even the published neonatal reference values show a variation depending on the kits used, the age at blood sampling, and the population studied (19–25). There are several studies published on the role of natriuretic peptides in the management of patent ductus arteriosus (26–30), persistent pulmonary hypertension of the newborn (31), as a marker of neonatal sepsis (32,33), and of left ventricular function (32,34). Previous investigations did neither show differences of NT-proBNP levels between arterial and venous cord blood nor a gender-related difference of NT-proBNP in neonates and children (22–24). Previously published NT-proBNP concentrations in the cord blood of healthy neonates and their mothers showed no correlation indicating that there is no placental exchange of NT-proBNP (22,23). NT-proBNP values in the cord blood of healthy neonates at time of delivery are elevated compared with older age groups and show a further increase after birth. They peak on the first to second day of life and constantly decrease beyond the second day of life (19–21,25). This elevation at birth is assumed to be a result of the dramatic circulatory changes during transition from fetal to neonatal life. With birth, the function of gas exchange is transferred from the placenta to the lungs. In healthy individuals, the venous and arterial circulation is separated, and from then on, cardiac output is equally divided between two ventricles. Congenital cardiac anomalies may affect the fetal circulation and normal adaptation after birth (35,36). So far, there are limited data published on NT-proBNP levels in the cord blood of neonates with congenital heart defect (37).

The purpose of this prospective study was to test the hypothesis that NT-proBNP is elevated in the cord blood of neonates with congenital heart defect compared with healthy neonates. Furthermore, this study investigates whether NT-proBNP decreases beyond the second day of life in neonates with congenital heart defect as it is reported in healthy neonates.

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Abbreviations: BNP, brain-type natriuretic peptide; CHD, congenital heart defect; NT, proBNP, amino terminal pro brain-type natriuretic peptide; r^2 , correlation coefficient

Table 1. Population characteristics

	CHD (<i>n</i> = 60)	Control group (<i>n</i> = 200)
Maternal age (y)	30.3 (19.5–41.3)	29 (17–42)
Gestational age (wk)	38 (35–40)	39 (37–42)
Male neonates	43 (72%)	100 (50%)
Birth weight (g)	3030 (1600–3580)	3348 (2240–4690)
Cesarean section	21 (35%)	60 (31%)
Apgar score		
At 1 min	9 (4–10)	9 (4–9)
At 5 min	9 (6–10)	10 (7–10)
At 10 min	10 (8–10)	10 (9–10)
pH		
Arterial	7.27 (6.99–7.47)	7.32 (7.03–7.45)
Venous	7.30 (7.15–7.47)	7.32 (7.09–7.58)

n, number.

PATIENTS AND METHODS

Congenital heart defect group. From May 2005 to January 2008, NT-pro BNP levels in the umbilical cord blood of 60 neonates with prenatally diagnosed congenital heart defect (CHD) at time of delivery and in the plasma of their mothers were measured using an automated enzyme immuno assay (Elecsys 2010, Roche Diagnostics). Fifty-three patients had ductus dependent lesions and 36 had a univentricular heart defect. The population characteristics of the patient group and the control group are shown in Table 1. The most common diagnosis was transposition of the great arteries (*n* = 10) in patients with biventricular heart defect, and hypoplastic left heart syndrome (*n* = 25) in patients with univentricular heart defect.

One patient with severe Ebstein anomaly died on the 5th day of life due to heart failure. Five patients did not need neonatal heart surgery (one patient with single ventricle, one with tricuspid atresia, two patients with tetralogy of Fallot, and one patient with congenitally corrected transposition of the great arteries). The remaining 54 patients with CHD underwent neonatal heart surgery. In this CHD subgroup, blood was drawn preoperatively on the median 5th day of life (range, 3–10 d) and NT-proBNP levels were additionally analyzed.

Control group. To serve as controls, the NT-pro BNP levels in the umbilical cord blood of 200 healthy neonates (100 males and 100 females) at time of delivery and in the plasma of their healthy mothers were analyzed during a 3-month period from March 2008 to July 2008. In a physical examination by the neonatologist and a routinely performed ultrasound of the kidneys on the second to 5th day of life, all neonates in the control group were considered to be healthy. The history of all mothers was unremarkable; mothers with any abnormality, such as gestationally induced diabetes or arterial hypertension were excluded from the study.

Because of the policy of our perinatologists to induce labor in the 38th week of gestation in fetuses affected with congenital heart defect, the CHD group showed a significantly lower gestational age and birth weight than the term born control subjects. Signs of fetal distress (reflected by Apgar score and umbilical pH), mode of delivery, and maternal age did not differ in the two groups (Table 1).

The Upper Austrian ethic's committee approved this study and informed consent was obtained from the guardian of each subject enrolled in the study.

Statistical analysis. Puncture of either the umbilical artery or the umbilical vein was technically not performed successfully in 14 subjects of the CHD group and in 45 subjects of the control group. These missing values of umbilical pH were not replaced; all other data were complete. All metric variables were tested for a normal distribution (test of normality: Kolmogorov-Smirnov with Lilliefors significance correction, type I error = 5%). For the comparisons of normally distributed variables between the cohorts and subgroups, the *t* test for independent samples was used. Not normally distributed metric data were analyzed with the Mann-Whitney *U* test. Within the cohorts not normally distributed, metric variables were compared by the dependent Wilcoxon test. Spearman's rank correlation coefficients were calculated for not normally distributed metric variables. All tests are two-tailed with a confidence level of 95% (*p* < 0.05). Data are reported as median with range.

RESULTS

Control group. The NT-proBNP levels in the cord blood of healthy neonates at time of delivery were median (range) 626

pg/mL (153–2518 pg/mL). NT-proBNP levels did not differ between male and female neonates in the control group [median (range): 640 pg/mL (153–2518 pg/mL) and 607 pg/mL (214–2346 pg/mL); *p* value: 0.42]. The NT-proBNP concentrations for the newborns and their respective mothers did neither correlate in the control group [median (range): 66 pg/mL (5–368 pg/mL); *r*²: 0.11] nor in the CHD group [median (range): 71 pg/mL (10–2191 pg/mL); *r*²: –0.17].

Congenital heart defect group. NT-proBNP levels were significantly higher in the cord blood of neonates affected with CHD [median (range): 1589 pg/mL (378–7626 pg/mL)] compared with healthy neonates [median (range): 626 pg/mL (153–2518 pg/mL); *p* value <0.001] (Fig. 1). Further subgroup analysis of the CHD group showed that NT-proBNP levels in the umbilical cord blood of patients with univentricular heart defects were significantly higher compared with patients with biventricular heart defects [median (range): 2001 pg/mL (378–7626 pg/mL) and 962.5 pg/mL (443–2585 pg/mL); *p* value <0.001].

In 54 patients with CHD, who underwent neonatal open-heart surgery, the preoperative NT-proBNP levels on the median 5th day of life (range, 3–10 d) were analyzed. NT-proBNP showed an increase compared with the cord blood levels in every single patient [median (range): 1665 pg/mL (378–7626 pg/mL) and 19222 pg/mL (1429–172800 pg/mL);

Figure 1. Box plots of NT-proBNP concentrations in the umbilical cord blood of neonates with CHD compared with healthy neonates.

Figure 2. Box plots of NT-proBNP concentrations of neonates with CHD in their cord blood and on the median 5th day of life.

p value <0.001] (Fig. 2). To exclude renal impairment, creatinine levels in the CHD group were measured on the median 5th day of life in all patients. Creatinine levels were within the normal range for age with median (range) 0.42 mg/dL (0.21–0.85 mg/dL).

One mother in the congenital heart defect group showed a markedly elevated NT-proBNP level of 2191 pg/mL. Further medical examination of this mother revealed pulmonary embolism due to factor V Leiden mutation.

DISCUSSION

The major new finding of this study is that NT-proBNP levels in the umbilical cord blood of neonates with congenital heart defect at time of delivery are significantly elevated compared with healthy neonates. Neither in the CHD group nor in the control group, the NT-proBNP umbilical cord blood levels correlated with the plasma concentrations of the respective mothers. This clearly indicates that the fetal heart on its own is able to secrete NT-proBNP in response to altered loading conditions. This hormonal activation might be either the result of immoderate up-regulation of genes in the fetal heart affected with CHD (38) or it reflects the fact that the circulatory anomalies in the fetus and the hemodynamic changes during labor contribute to an overexposure to myocardial stretch in congenital heart defect patients, leading to excessive secretion of NT-proBNP (35,36). The highest cord blood levels were found in patients with univentricular heart defects, suggesting that maintenance of cardiac output by a single ventricle leads to volume overload of this ventricle and subsequently to an increased secretion of natriuretic hormones. Although we found a significant difference between the cord blood NT-proBNP levels of healthy neonates compared with neonates with CHD, a single NT-proBNP measurement in the cord blood cannot separate healthy neonates from neonates with CHD due to the marked overlap between both groups (Fig. 1).

Caused by perinatal circulatory changes and the immaturity of the kidneys, NT-proBNP concentrations in healthy neonates show a marked increase after birth and peak on the first to second day of life. Beyond the second day of life, maturation of the renal function leads to a steady decrease of NT-proBNP levels in healthy infants (19–21,25).

The second important finding of our study is that in patients with CHD NT-proBNP concentrations remain markedly elevated beyond the second day of life. It remains unknown, whether this elevation is due to the volume overload observed with prostaglandin induced open arterial ductus or it is genuinely present in neonates with complex CHD.

This study confirms in a significant number of subjects the previously reported finding (21–24) that NT-proBNP in the umbilical cord blood of healthy neonates is elevated compared with the reference interval for older age groups. This assumedly physiologic elevation in healthy neonates might be explained by the changes in ventricular volume load and ventricular stroke volume during transition from fetal to neonatal life. Using the same assay, Bar-Oz *et al.* (23) reported a mean umbilical cord blood concentration in healthy neonates

of 600 pg/mL, and Bakker *et al.* (24) found a mean concentration of 80 pmol/L (670 pg/mL). Both reports are well comparable to our results of median 626 pg/mL in the healthy control group. In agreement with previous publications our study confirms that NT-proBNP levels in umbilical cord blood show no gender-related difference (21–24).

In conclusion, NT-proBNP levels in the umbilical cord blood of neonates with congenital heart defect are significantly elevated at time of delivery compared with healthy neonates. Additionally, NT-proBNP concentrations in neonates with CHD significantly increase beyond the second day of life.

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