Letter to the Editor

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Amino-terminal proB-type natriuretic peptide reference values in umbilical cord blood

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To the Editor,

The brain natriuretic peptide (BNP) and the N-terminal part of its prohormone (amino-terminal proB-type natriuretic peptide; NT-proBNP) are secreted from cardiac atrium and ventricular myocytes in response to an increase in cardiac wall stretch, volume and pressure loading, as wells as in hypoxia. Both of them are well-accepted markers in the process of diagnosis, prognosis and treatment of cardiac disease in adult population [1], but the higher plasma half-life of NT-proBNP than the BNP (120 vs. 20 min), as well as its higher stability at room temperature, makes NT-proBNP the preferred molecule to be analysed for use in clinical practice [2]. Although heart disease is not as common in children as in adults, it is a significant cause of morbidity and mortality in infants and children. In the field of paediatric cardiology, several studies have assessed NT-proBNP concentrations in various foetal and neonatal conditions associated with foetal cardiovascular dysfunction (congenital heart disease, patent ductus arteriosus, pulmonary hypertension, hydrops or respiratory distress) [3, 4], as well as in several pregnancy-related pathologies (maternal diabetes, pre-eclampsia, foetal growth restriction, preterm birth and foetal distress) [5, 6]. As a result, NT-proBNP is considered to be a very sensitive marker of foetal and neonatal cardiac stress, not affected by maternal factors [6]. Therefore, cord blood NT-proBNP concentrations could be a potential tool in the early diagnosis and associated treatment of neonatal diseases with related cardiovascular impairment. However, to facilitate NT-proBNP use in paediatric cardiology, a reliable reference interval is mandatory. A recent update on the application of NT-proBNP as a biomarker of myocardial dysfunction during foetal life shows that most of the previous studies compared NT-proBNP concentrations between affected and a small sample of healthy neonates, and only a few studies have focused on establishing reference values in cord blood of healthy neonates, most of them with a sample size smaller than 250 [7–9].

Taking into consideration the lack of consensus on the reference values and the maternal and foetal factors that can affect cord blood NT-proBNP concentrations, we obtained NT-proBNP reference values in the cord blood of a representative healthy population of neonates. Umbilical cord blood was collected in sterile tubes without any anticoagulant (BD Vacutainer; BD Vacutainer® SST™ II Advance 8.5 mL, Ref. 366468), centrifuged at 5098 × *g* for 5 min and the supernatant was immediately separated and stored at −80 °C until assayed. We measured NT-proBNP concentrations in umbilical cord blood from 281 neonates using an electrochemiluminescence sandwich immunoassay (Cobas 6000 analyser; Roche Diagnostics; proBNP II, Ref. 04842464190), after checking all medical records until 2 years after birth and excluding all cases with hypoxia, respiratory distress, neonatal cardiopulmonary resuscitation, intrauterine foetal growth restriction (IUFGR) and any cardiac pathology. The following data related to pregnancy, delivery and birth were collected: gestational age, mode of delivery, neonatal sex and weight, cord blood pH and Apgar score at 1 and 5 min. Demographic characteristics of the included population are shown in Table 1. Outliers were detected using the Tukey test and excluded (n = 12). The categorical variables were expressed as numbers and percentages with a confidence interval of 95% (CI 95%), while the quantitative variables were expressed as a median with a minimum and maximum, or a mean with standard deviation. We

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Table 1: Characteristics of the reference population.

	n (%)	x ± SD or median (min–max)
Birth weight, g	269	3296 ± 423
Gestational age, weeks	269	40 (35–42)
Prematurity, weeks		
Premature (27– < 37)	14 (5%)	
Term (37–41)	249 (93%)	
Post-term (≥42)	6 (2%)	
Sex (M/F)	145 (54%)/124 (46%)	
Mode of delivery		
SVD	177 (66%)	
Caesarean section	20 (7%)	
Vacuum/forceps	72 (27%)	
1-min Apgar score		9 (4–10)
5-min Apgar score		9 (4–10)
Cord blood pH		7.27 ± 0.09
PROM	90 (33%)	

Normally distributed variables are presented as mean ± standard deviation and all others as median (range). SVD, spontaneous vaginal delivery; PROM, premature rupture of membranes; n, number of cases; x ± SD, (mean ± standard deviation); min, minimum; max, maximum; M/F, (male/female).

used the Mann-Whitney U test (paired groups) or the Kruskal-Wallis test (≥2 groups), with Bonferroni correction, to compare NT-proBNP concentrations with the following parameters: neonate sex, type of delivery, premature rupture of membranes (PROM) and gestational weeks (premature: 28–37 weeks; term: 37–41 weeks; post-term: ≥42 weeks). The Spearman correlation was applied to evaluate the relationship between NT-proBNP concentration and continuous variables: umbilical cord pH, gestational weeks, birth weight, 1- and 5-min Apgar scores. A p-value ≤0.05 was considered statistically significant. As a non-Gaussian distribution of NT-proBNP concentrations was obtained (D'Agostino-Pearson test), reference interval, defined as the interval of values between the 2.5th and 97.5th percentiles, was calculated following the Clinical and Laboratory Standards Institute (CLSI) guideline C28-A3, by the non-parametric percentile method, using SPSS 15.0 and MedCalc (v 12.5.0.0). The study was approved by the local Ethics Committee (Principado de Asturias Research Ethics Committee; approval number 276/19) with exemption from informed consent.

NT-proBNP concentrations showed no significant association with regard to neonatal sex (male or female) and gestational age (premature, term or post-term neonates), whereas a weak negative correlation was observed between NT-proBNP concentrations and birth weight (rho = –0.164, $p < 0.01$). Considering the type of delivery, NT-proBNP concentrations were significantly lower in spontaneous vaginal delivery (SVD) (524 pg/mL [178–2632]) than in non-SVD (caesarean section: 713 pg/mL [390–2178]; vacuum/forceps: 654 pg/mL [240–2877]; $p < 0.01$). On the other hand, there were 90 cases of PROM (33%), which showed NT-proBNP concentrations significantly higher than those in non-PROM deliveries (690 pg/mL [210–1999] vs. 551 pg/mL [178–2877]; $p < 0.01$). If we consider neonatal status, no correlation was found between NT-proBNP concentrations and umbilical cord blood pH. However, NT-proBNP cord blood concentrations showed a weak negative association with 1- and 5-min Apgar scores (rho = – 0.202, $p < 0.01$ and rho = – 0.149, $p < 0.05$, respectively).

Regarding the NT-proBNP reference values, the lower reference limit for all neonates was 240 pg/mL (CI 95% = 221–263) and the upper limit was 1985 pg/mL (CI 95% = 1638–2270). Reference limits taking into account PROM and the type of delivery are shown in Table 2.

We determined cord blood NT-proBNP reference values in a representative healthy population of neonates, by a standardised protocol, and described the relation of NT-proBNP concentrations to birth weight, Apgar score and the influence of the mode of delivery and PROM. There has been a wide variability in cord blood NT-proBNP reference values described to date [7]. Taking into account the same technology used in our study (Elecsys Roche technology), only Kocylowski et al. [8] have published umbilical cord blood NT-proBNP reference ranges in a high population of healthy newborns (n = 278), but stratifying values according to weeks of gestation and calculating the reference interval not following the CLSI recommendations. Regarding the maternal and neonatal factors that could influence cord blood NT-proBNP concentrations, the negative

Table 2: NT-proBNP concentrations (pg/mL) of the reference population.

	n	Median (min–max)	Lower limit (CI 95%)	Upper limit (CI 95%)
All neonates	269	575 (178–2877)	240 (221–263)	1985 (1638–2270)
Non-PROM neonates	179	551 (178–2877)	234 (178–261)	1858 (1340–2877)
SVD	177	524 (178–2632)	234 (178–261)	1882 (1295–2632)
Non-SVD[a]	92	662 (240–2877)	258	2040

[a]Confidence interval is not calculated because of the sample size. SVD, spontaneous vaginal delivery; PROM, premature rupture of membranes; n, number of cases; min, minimum; max, maximum; CI, confidence interval.

association between NT-proBNP concentrations and birth weight, as well as for 1- and 5-min Apgar scores, is thought to be related to a stressful intrauterine environment that induces the foetus to produce large amounts of NT-proBNP, explaining its role in maturing the cardiovascular system and reflecting renal and pulmonary maturation. Our results in this aspect support those previously described by Seong et al. in a smaller population [10]. Similarly, the increase in NT-proBNP concentrations during non-SVD delivery could also be a response to a stressful situation of the foetus, as vaginal delivery is not associated with elevation of NT-proBNP concentrations [10]. Moreover, little is known about NT-proBNP cord blood concentrations in PROM, another potential stressful foetal situation, where we have found higher concentrations of NT-proBNP than in non-PROM pregnancies.

The availability of a reliable NT-proBNP cord blood reference range in our own population, measured with a widely used technology, as well as the knowledge of the factors that can vary its concentrations, will facilitate its implementation and use in clinical practice as a marker of volume overload in neonatal pathological situations.

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