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B-type natriuretic peptide reference interval of newborns from healthy and pre-eclamptic women: a prospective, multicentre, cross-sectional study

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ABSTRACT

Objective To define and compare the reference interval of B-type natriuretic peptide (BNP) in healthy newborns (HN) from healthy mothers and with severe pre-eclampsia.

Design Prospective, multicentre, cross-sectional study.

Setting Four obstetric wards of second-level academic hospitals.

Participants 167 HN, from 146 healthy and 21 severe pre-eclamptic women. We included newborns from healthy mothers with full-term pregnancies (38 to 42 gestational weeks), who received adequate prenatal care and who had Apgar scores ≥7 at 0 and 5 min. Newborns with chromosomopathies identified during prenatal consultations, those with respiratory distress and those with cardiac or pulmonary disease detected in the first paediatric evaluation were excluded from this study. In the group of pre-eclamptic women, we considered the same inclusion criteria, but the patients also had to meet the diagnostic criteria for pre-eclampsia with severity features, according to the American College of Obstetricians and Gynaecologists guidelines. The same exclusion criteria used for the healthy group were applied to the pre-eclampsia-associated newborn.

Interventions A single blood sample from the umbilical cord artery after delivery (vaginal or caesarean section).

Primary outcome Reference level of BNP in HN.

Results In the HN group, the median BNP was 12.15 pg/mL (IQR 7.7–16.8 pg/mL) and in the pre-eclamptic group 20.8 pg/mL (IQR 5.8–46.5 pg/mL). The reference interval for BNP in HN was 5pg/mL (95% CI 5 to 5) to 34 pg/mL (95% CI 28.4 to 38.8). We identified higher expression of BNP in newborns from pre-eclamptic women overall (p=0.037, r=0.16) and in newborns exposed to stress conditions, such as complications during labour and delivery (p=0.004, r=0.33).

Conclusions In HN, BNP concentrations at birth were lower than reported in other similar populations. In neonates with stress conditions, the higher expression of this biomarker establishes another possible link between stress and the cardiovascular response.

Trial registration number NCT02574806; Pre-results.

Strengths and limitations of this study

- ► First prospective multicentre cohort study aimed to determine the reference interval of B-type natriuretic peptide (BNP) in the healthy newborn after delivery.
- ► Study population, sample size and statistical analysis were defined according to the guidelines from the Clinical & Laboratory Standards Institute.
- ► There were no serial measurements of BNP for follow-up.
- ► The study is limited only to BNP, due to its relevance as a marker of ventricular dysfunction.
- ► No imaging tools were used for structural and functional assessment.

INTRODUCTION

Several studies have proven the B-type natriuretic peptide (BNP) as a promising diagnostic and stratification biomarker in multiple clinical settings concerning the adult population.[1] In paediatric patients with cardiac disorders, BNP is an essential tool for diagnosis, risk stratification and prognosis,[2] since elevated expression of BNP in the paediatric population has been associated with poor ventricular function and outcomes as seen in patent ductus arteriosus, persistent pulmonary hypertension of the newborn and stress transient cardiomyopathy.[3 4] However, newborns have peak levels in the first days of life, which decrease within the first week and vary greatly.[5] The published normal range of BNP and the amino-terminal segment of its prohormone (N-terminal pro-BNP (NT-pro-BNP)) appear to have an age-dependent and gender-dependent trend.[6] The ample range of blood concentrations of both biomarkers can be attributed to several perinatal factors that may be influencing myocardial performance during the postnatal transition period.[5 7–10] Despite aforementioned data,[3 4] perhaps BNP is not the best option as a biomarker in neonates.[6] On the other hand, in newborns with acute respiratory distress, a normal BNP value may have

a high negative predictive value for ruling out ventricular dysfunction from other acute disorders (ie, respiratory distress, sepsis or metabolic alterations), improving the quality of medical care. Additionally, pre-eclampsia is a vascular disorder that increases maternal and fetal/neonatal morbidity and mortality,[11] increases BNP expression[12] and has deleterious effects on the cardiovascular system of the newborn.[13] Whether these maternal mechanisms influence the expression of BNP in the newborn is an unmet question. Currently, BNP concentration has an impact in early disease stratification. Nonetheless, its role and the normal concentrations in the healthy neonatal population have not been elucidated. Also, the influence of maternal pre-eclampsia on the cardiovascular system of the newborn is not well known. Therefore, we conducted the first prospective, multicentre, cross-sectional study to establish the reference interval of BNP in healthy newborns (HN), and thereafter compared BNP concentrations between newborns of healthy and pre-eclamptic mothers.

METHODS

Study design

A multicentre, prospective, cross-sectional study was designed to establish the reference interval of BNP in HN at birth and to compare it with those in offspring of pre-eclamptic mothers. The study was conducted at the obstetrics wards of four academic hospitals, from April 2014 to April 2015. Medical health records were reviewed to assess the potential eligibility of each participant, and a direct interview confirmed that all inclusion criteria were met. Inclusion criteria newborns: (1) from healthy mothers with full-term pregnancies (38 to 42 gestational weeks), (2) who received adequate prenatal care and (3) who had Apgar scores ≥7 at 0 and 5 min. Exclusion criteria were (1) newborns with chromosomopathies identified during prenatal consultations, (2) those with respiratory distress and (3) those with cardiac or pulmonary disease detected in the first paediatric evaluation. In the group of pre-eclamptic women, we considered the same inclusion criteria, but the patients also had to meet the diagnostic criteria for pre-eclampsia with severity features, according to the American College of Obstetricians and Gynaecologists (ACOG) guidelines. The same exclusion criteria used for the HN group were applied to the pre-eclampsia-associated newborn (PAN) group (figure 1).

We collected a single whole blood sample from the umbilical cord artery after delivery (vaginal or caesarean section) in 4 mL spray-coated K2EDTA tubes (BD Vacutainer), which was immediately processed and analysed, eliminating need for storage. Arterial blood was preferred over venous blood because this would allow a direct measurement of BNP concentration from the bloodstream of the newborn. After determining the BNP reference interval in HN, this group was compared with newborns from mothers with severe pre-eclampsia. Anthropometry and clinical status were obtained from the records of attending paediatricians and neonatologists. All infants were evaluated in the nursery ward during the first hours of the neonatal period to ascertain their health status. Written informed consent was obtained from all mothers before newborns enrolment. This study was conducted according to the Declaration of Helsinki. The trial was registered in ClinicalTrials.gov: NCT02574806.

BNP measurement

BNP was measured by using the Triage MeterPlus and Triage BNP Test (Alere). The Triage BNP assay is a rapid, point-of-care fluorescence immunoassay for the quantitative measurement of BNP in potassium EDTA-anticoagulated whole blood or plasma specimens.[14] In brief, the sample reacts with fluorescent antibody conjugates and flows through the test device by capillary action. Complexes of fluorescent antibody conjugate are captured in a specific area for BNP. BNP concentration in the specimen is directly proportional to the fluorescence detected, and in approximately 15 min, a result is obtained. The measurement range of the BNP assay is 5 pg/mL to 5000 pg/mL. A measurement range >100 pg/mL is determined as abnormal by the equipment referencing literature.[14]

Study definitions

A healthy mother was considered as a biological woman between 15 and 36 years old, without chronic disease history or pregnancy-associated diseases. Adequate prenatal care was defined using the WHO criterion, consisting of at least five obstetric office visits. Severe pre-eclampsia was diagnosed according to the ACOG criteria which consist of: the presence of pre-eclampsia with systemic involvement, including heart failure, visual or cerebral disturbances, thrombocytopenia, abnormal liver tests and/or renal failure.[15] Complicated delivery was defined as prolonged labour (≥14 hours), evidence of abnormal antenatal cardiotocographic records, no reassuring fetal status, amniotic fluid abnormalities (ie, volume disturbances, meconium), aspiration of meconium during birth or umbilical cord compression.

Statistical analysis

The sample size was determined based on the guidelines from the Clinical & Laboratory Standards Institute, which establishes that the minimum sample of healthy reference subjects required to obtain a reference interval with a 95% CI is 146 subjects.[16–18] Continuous variables were expressed as mean±SD. Furthermore, categorical variables were expressed as numbers and percentages. All values are rounded to the nearest 10th decimal. Values of BNP were shown as median and IQR. The reference interval of BNP in HN was determined by estimation of the 2.5th and 97.5th percentile with 95% CI, by parametric or non-parametric methods depending on BNP values distribution, as recommended by Clinical & Laboratory Standards Institute.[17] For non-parametric continuous variables, we used Mann-Whitney U test to

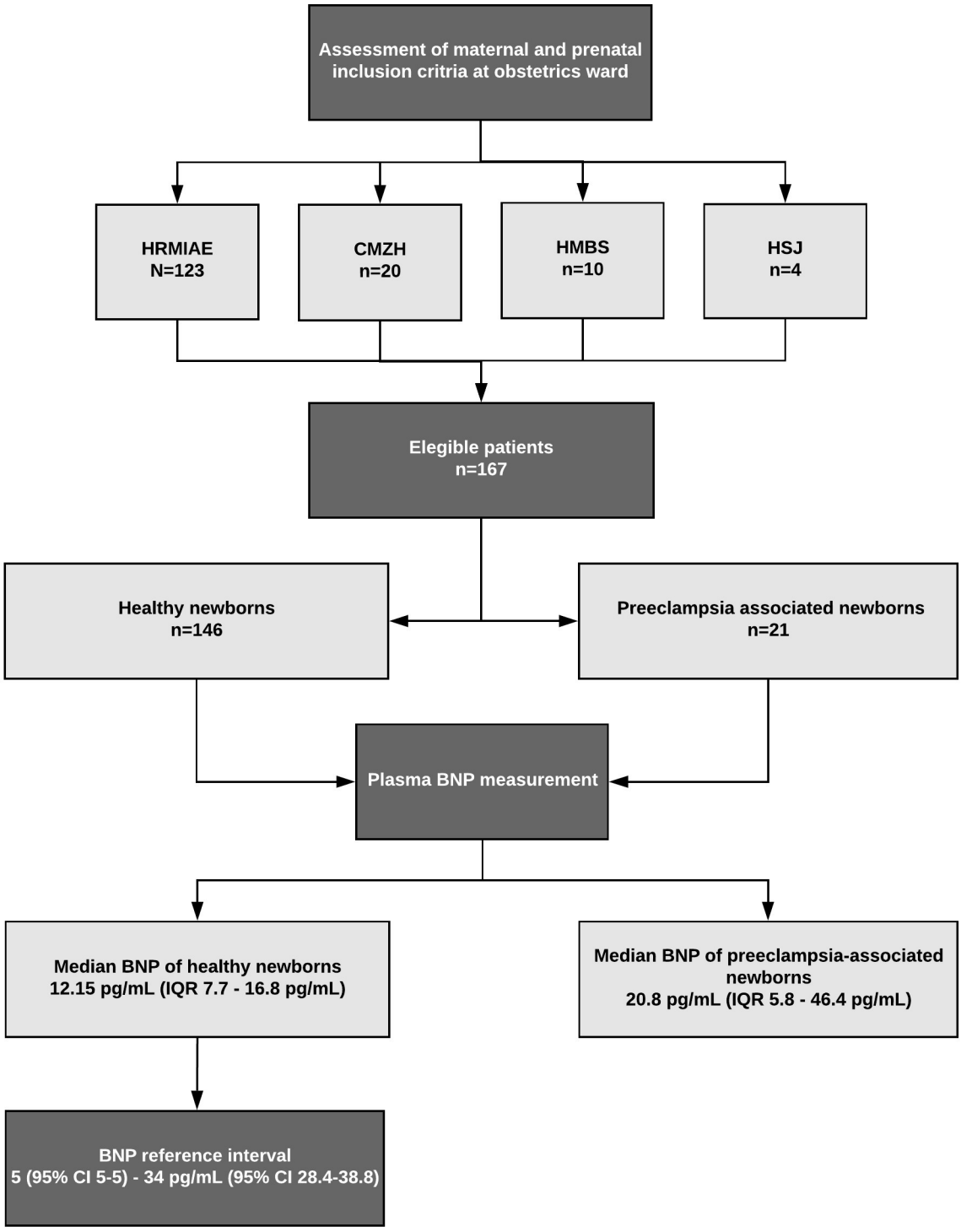

Figure 1 Flow diagram of study and B-type natriuretic peptide (BNP) values.

compare BNP expression between groups. Chi-square tests for association were conducted to evaluate percentage differences between categorical variables among newborns from HN and PAN groups. A probability of alpha-error <0.05 was considered for significant results. Statistical analysis was performed using the Statistics Package for the Social Sciences (SPSS) from IBM Corporation, V.22, and Stata/IC V.14.2 for Mac (College Station, Texas, USA). Graphs were made with Prism V.7 for Mac OS X.

Patient involvement

No patients were involved in the planning of this research.

RESULTS

From April 2014 to April 2015, 167 samples were collected in two hospitals from the public sector and two from the private sector; 146 newborns were categorised as the healthy group, and 21 as the pre-eclampsia group. The latter group was composed of newborns

Table 1 Demographic and clinical characteristics of healthy and pre-eclamptic mothers and newborns

Characteristics	Healthy group, n=146 Median [Q1–Q3], No. (%)	Pre-eclamptic group, n=21 Median [Q1–Q3], No. (%)	P values
Mothers			
Age (years)	23.5 [18–29]	21 [18–28]	0.48
Public sector	118 (80.8)	21 (100)	0.026
Gravidity >1	76 (52)	9 (43)	0.43
Abortion >1	4 (2.7)	1 (4.8)	0.61
Gestational age (weeks)	39.5 [38.5–40.2]	38.4 [37.2–40.2]	0.005
Spontaneous labour	76 (52)	14 (66.7)	0.20
Spontaneous ruptures of membranes	87 (59.6)	17 (81)	0.058
Epidural analgesia	141 (96.6)	20 (95.2)	0.75
Normal delivery	79 (54.1)	3 (14.3)	0.0007
Newborns			
Male	79 (54.1)	6 (28.6)	0.02
Apgar score at 0 min	8 [8–9]	8 [8–8]	0.014
Apgar score at 5 min	9 [9–9]	9 [9–9]	0.005
Weight (kg)	3.3 [3.0–3.5]	3 [2.7–3.3]	0.009
Estimated gestational age by Dubowitz score (weeks)	39.2 [38.4–40.1]	38.3 [37–39.6]	0.005
Complicated delivery	23 (15.8)	7 (33.3)	0.049
Cyanosis	0	5 (23.8)	0
Respiratory distress	0	3 (14.2)	0.0017
Nursery	146 (100)	18 (85.7)	0.0017
Neonatal intensive care unit	0	3 (14.2)	0.0017

from mothers who developed pre-eclampsia during the third trimester of pregnancy, requiring admission to the obstetric intensive care unit (table 1). Maternal population was younger, with all HN delivered at term, and high incidence of spontaneous labour and rupture of membranes (table 1). Both the HN and PAN groups had similar proportions of nulliparous and multiparous mothers and small percentages of previous miscarriages or abortions. A high rate of newborn deliveries in HN and PAN groups were performed by caesarean section. We observed delivery complications in 15.8% and 33.3% neonates from the HN and PAN group, respectively. From the PAN group, 24% neonates presented cyanosis and 14.2% respiratory distress. There were no missing data.

A non-normal distribution of BNP values was found in both study groups by visual histogram inspection and the Shapiro-Wilk test (p<0.05) (figure 2). Therefore, we decided to follow a non-parametric analysis approach to determine the reference interval and assess group differences in BNP concentrations. In the healthy group, the BNP median was 12.15 pg/mL (IQR 7.7–16.8 pg/mL) and in the pre-eclamptic group 20.8 pg/mL (IQR 5.8–46.4 pg/mL). The reference interval of BNP in HN based on 2.5th and 97.5th percentiles was 5 pg/mL (95% CI 5 to 5) to 34 pg/mL (95% CI 28.4 to 38.8) (figure 3). Offspring from the PAN group had significantly higher BNP levels compared with those in the HN (P=0.037), with a small effect size, r=0.16. Almost one-third of the PAN group (28.6%) were outside the reference range. Figure 3 shows BNP concentrations in HN and PAN

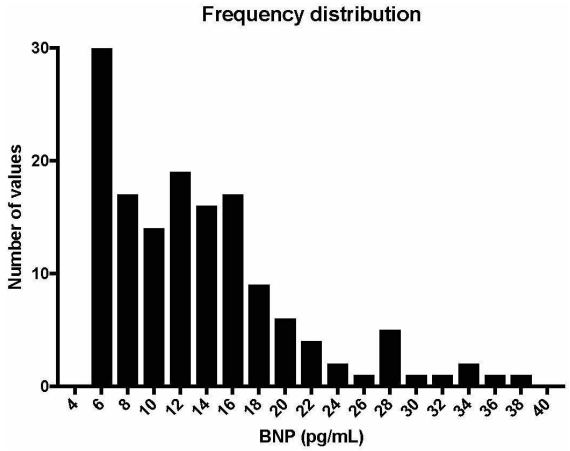

Figure 2 The distribution of the B-type natriuretic peptide (BNP) values in healthy newborn group. The right-skewed distribution was related to the limit of quantification of the equipment (5 pg/mL). Thus, a logarithmic transformation was performed. However, the distribution remains right-side skewed. Consequently, the reference interval was estimated using the rank-based method for non-parametric distribution.

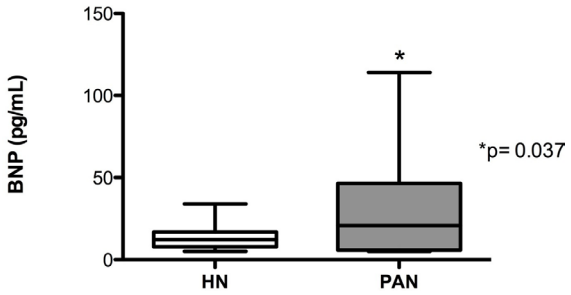

Figure 3 Box plot of reference interval of B-type natriuretic peptide (BNP) values in the healthy newborn (HN) and pre-eclampsia-associated newborn (PAN) group (whiskers represent 2.5th and 97.5th percentiles).

groups. Additionally, subgroup analyses of HN group are shown in table 2. Also, we described medians and IQR of BNP in the same table. Children from the HN group delivered by caesarean section had significantly higher (p=0.18) BNP values than those born by vaginal delivery, although with a small effect size, r=0.19 (median 13.8 pg/mL, IQR 8.9–18.1 pg/mL vs 11.2 pg/mL, IQR 6.8–15.3 pg/mL, respectively). The Mann-Whitney U test that evaluated BNP concentrations between newborns in the PAN group with and without delivery complications was also significant, in the expected direction (p=0.038), and with a moderate effect size, r=0.45. Those babies with delivery complications had higher BNP values (median 47.9 pg/mL (IQR=12.2–62.6 pg/mL)) compared with the ones with non-complicated deliveries (median 15.5 pg/mL (IQR=5.0–25.4 pg/mL)). Also, we identified that complicated deliveries in the PAN group had higher expression of BNP than those babies with complicated delivery in the HN group (p=0.004, r=0.33).

Lastly, χ^2 tests for association were conducted to evaluate percentage differences in labour initiation, a method of membrane rupture, the use of analgesia during delivery, development of delivery complications, the presence of cyanosis or respiratory distress and admission to the neonatal intensive care unit (NICU) among newborns from HN and PAN groups. Analyses showed no significant association with spontaneous or induced labour initiation (p=0.209), the method of membrane rupture (p=0.059), use of analgesia during labour (p=0.75) nor for the presence of delivery complications (p=0.109) with HN or PAN groups. However, significant association was found between newborns born from pre-eclamptic mothers and the presence of cyanosis ($x^2(1)=3583$, p=0.000, Cramer's V=0.46), the development of respiratory distress ($x^2(1)=21.23$, p=0.000, Cramer's V=0.35) and

Table 2 Subgoup analysis of median B-type natriuretic peptide of HN and PAN groups

	Healthy newborns		Pre-eclampsia-associated newborns
	BNP Median (Q1–Q3)	P values	BNP Median (Q1–Q3)
Overall BNP	12.1 (7.7–16.8)	–	20.8 (5.8–46.4)
Sex			
Male	12.0 (7.5–16.9)	0.91	11.15 (5.0–49.5)
Female	12.2 (8.4–16.0)		22.3 (9.9–45.1)
Delivery			
Spontaneous	11.8 (7.7–16.0)	0.56	21.5 (11.6–36.4)
Induced labour	12.5 (8.3–17.4)		6.3 (5.0–57.6)
Analgesia			
Epidural analgesia	11.8 (7.7–16.6)	0.08	18.4 (5.5–41.9)
Non-analgesia	14.3 (12.7–27.7)		54.5 (54.5–54.5)
Delivery method			
Vaginal delivery	11.2 (6.8–15.3)	0.01	32.6 (15.1–32.6)
Caesarean section	13.8 (8.9–18.1)		18.4 (5.2–45.7)
Membrane rupture			
Spontaneous	11.7 (7.2–15.8)	0.05	20.8 (8.1–38.8)
Artificial rupture of membranes	13.8 (8.7–19.2)		31.3 (5.0–61.3)
Complications			
Delivery complications	8.9 (6.7–13.7)	0.11	47.9 (12.2–62.6)
Normal delivery	12.3 (8.3–16.8)		15.5 (5.0–25.4)

The overall B-type natriuretic peptide (BNP) row describes the comparison of the median BNP measurement of the healthy newborns (HN). Also, the median and IQR range of BNP from pre-eclampsia-associated newborn (PAN) group.

admission to NICU ($x^2(1)=21.23$, p=0.000, Cramer's V=0.35).

DISCUSSION

The results from this multicentre study provide three important observations regarding the use of BNP concentrations in the paediatric population. First, BNP concentrations were lower in healthy offspring population at birth, compared with those observed in similar populations and lower than the values detected in paediatric[7 19] and adult patients.[12] Second, higher BNP concentrations in newborns with two stressors (ie, pre-eclampsia and complicated delivery) contribute to elucidate another link between stress and cardiac response.[20] Lastly, high peak levels of BNP concentrations known to be present at birth, as a consequence of the physiological conditions of the newborn, were not observed in our HN group.

BNP and the inactive NT-pro-BNP levels are reasonably correlated, and either of them can be used in patient care. Although the usefulness of BNP concentrations in identifying different ventricular dysfunction stages or mortality risks in the adult[21] and paediatric populations[4 7 19] has been well established, studies in children are more challenging and remain limited than those in adults. One of the most important factors promoting this limitation is the lack of normative biomarker values in children.[19] Additionally, average adult BNP values vary depending on the assay method and patient demographics; whereas appropriate reference values are lacking in children.

Our results show that in HN at birth, BNP concentration was lower (median 12.15 pg/mL (IQR 7.7–16.8 pg/mL)) than that reported in other neonate populations (96 pg/mL (mean 134±130 pg/mL)) without disease.[7] Although, in this study, newborns included had signs and symptoms of a cardiovascular disease requiring urgent paediatric cardiology consult, and most had a cardiovascular illness with or without anatomic defect.[7] Most studies show high levels of BNP and NT-pro-BNP immediately after birth, decreasing during the third or fourth day of life.[22 23] Other reports indicate a tendency or significant decrease in BNP concentrations, suggesting that the peptide levels are dependent on age, the assay used for its measurement and possibly the gender.[10] Additionally, a BNP cut-off value of 170 pg/mL during the first 7 days of extrauterine life and value of 41 pg/mL for those older than 7 days but younger than 19 years old have been established.[7]

In our results, BNP expression was independent of maternal characteristics including age and obstetric history. However, we did find increased BNP values in babies delivered by caesarean section, but only in the HN group. This finding is in accordance with previous studies that have reported that BNP expression was dependent on delivery method.[22–24]

Even though the reason for higher NT-pro-BNP or BNP values soon after birth remains unknown, several mechanisms have been considered, its role in the homeostasis, increased body water, myocardium stiffness, etc.[7] Moreover, newborns may be less responsive to natriuretic peptides, so a higher secreted level may be required to promote the well-recognised natriuresis that commences within the first week of life. During this time, the precipitous decrease in pulmonary vascular resistance may also be promoted by natriuretic peptides.[7] On the other hand, our results suggest that perinatal factors related to postnatal transition do not have any influence on myocardial performance. Since current and strong evidences[25–27] support that BNP does not cross the placental barrier, these values only reflect the myocardial performance to extrauterine life.

The whole blood BNP concentrations (median 12.15 pg/mL, IQR 7.7–16.8 pg/mL) observed in our HN population were similar to BNP levels throughout pregnancy in healthy women,[12] suggesting that a BNP concentration >20 pg/mL in pregnant women and newborns may indicate ventricular stress and/or subclinical cardiac dysfunction. Currently, in adult patients, ventricular dysfunction is unlikely when BNP concentrations are <100 pg/mL. However, some studies have suggested that a cut-off value of 50 pg/mL, rather than 100 pg/mL, will increase its sensitivity and negative predictive value in adult or paediatric populations.[28] The clinical implications of our findings suggest that in newborns, a reference interval of 5 pg/mL (95% CI 5 to 5) to 34 pg/mL (95% CI 28.4 to 38.8) instead of <100 pg/mL could increase its sensitivity and the negative predictive value reducing the proportion of false positives. BNP expression superior to the obtained reference interval could be considered as a grey zone of this biomarker, without clinical relevance in early phases. However, if this abnormal expression could have further clinical implications is an unsolved question. It is possible that the lower BNP concentrations observed in our study, as suggested by other observations,[3 7 10 29 30] may depend on racial or genetic characteristics. Recently, results support this consideration based on higher BNP concentrations (32.02±3.37 pg/mL, mean±SE) compared with our HN population.[31] However, the mechanisms behind this finding remain as an unresolved question.

Another relevant result was higher BNP concentrations in neonates under two stressors (maternal pre-eclampsia and delivery complications), compared with HN (median 47.9, IQR 12.6–62.6 pg/mL, vs 8.9, IQR 6.7–13.7 pg/mL, p=0.004, r=0.33) despite a good adaptation to extrauterine life. In the short-term follow-up, we did not observe a clinical important difference, since BNP concentrations were not associated with need for a specialist consultation, NICU transfer, death or congenital cardiac disease. Although the stress role in cardiovascular disorders has been well established,[20] its participation in other paediatric clinical models[21] is still uncertain. Since there is an embryological link between neurological diseases and cardiomyopathy (common neuroectodermal origin), catecholaminergical overexpression in neuroendocrine disease may elicit an abnormal response in the myocardial adrenergic receptors. Thus, in early stages, if this overexpression is not sustained, cellular disarray could

induce higher BNP concentrations. In late stages, and under conditions of continued stress, this mechanism could be linked to several models of transient hypertrophic cardiomyopathy.[21]

Lastly, although despite previous evidence establishing different BNP concentrations depending on the type of delivery[22–24] and by the presence of pre-eclampsia,[2] our results did not reproduce these observations. Our study group only showed increased BNP concentrations in neonates who were born by caesarean section from healthy mothers. Also, no differences among delivery type and BNP concentrations were identified.

There are several potential limitations of our study. A major limitation was the lack of long-term follow-up of the offspring, with paediatric visits and blood draws to establish serial measurements of BNP which could help determine its prognostic value of BNP for cardiovascular conditions during different stages of growth and development along with its relevance for chronic diseases. However, our aim was only to focus on the assessment of BNP expression in the HN as a tool for clinical decision making in the compromised neonate. Additionally, maternal BNP concentrations were not obtained, nor were other biomarkers or imagining diagnostic tools used. The influence of the placenta on biomarker levels was not examined by this study, and the sample size of the newborns from mothers with pre-eclampsia is small compared with the HN group (21 vs 146). Thus, the reader should be cautious when interpreting our results since only large differences between the HN and PAN groups will be statistically significant. However, we identified that stress conditions (pre-eclampsia and complicated delivery) are triggers that increase BNP expression, but it will be necessary to evaluate this finding in more extensive studies focused on a pre-eclamptic population.

Clinical implications

Newborns at birth with respiratory distress present an extra challenge to most physicians because of a lack of a well-communicated history. Rapid evaluation is needed so that they can be referred quickly to the correct physician (ie, cardiologist or pulmonologist) to receive appropriate treatment. If our results are reproduced, BNP concentrations could have a great future as a biomarker. In our population, the upper limit of BNP expression in the HN is <40 pg/mL; if this result is reproduced in future studies, this value could be used to address the clinical suspicion about the aetiology of the acute respiratory distress shortening the time to diagnosis and undergo specialised treatment.

CONCLUSION

In HN, BNP concentration at birth was lower than previously reported in similar populations. Neonates under stress conditions related to complications at birth showed a higher expression of this biomarker, which elucidates a possible link between stress and the corresponding cardiovascular response in this population.

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Contributors DR and CJ-S contributed to the design and implementation of the protocol, the analysis of the results and the writing of the manuscript. GG-R and EL-S contributed to the analysis of the results and the writing of the manuscript. JY and GT-A contributed to the design of the protocol, the analysis of the results and the writing of the manuscript.

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Competing interests None declared.

Patient consent Guardian/consent obtained.

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Data sharing statement The authors do not have permission to share data since our informed consent form did not inquire for permission to share data.

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