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N-Terminal-pro-B Type Natriuretic Peptide as a Useful Tool to Evaluate Pulmonary Hypertension and Cardiac Function in CDH Infants

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Key Words
N-terminal-pro-B type natriuretic peptide · Congenital diaphragmatic hernia · Pulmonary hypertension · Cardiac function · Diastole

Abstract
Objective: In congenital diaphragmatic hernia (CDH) the severity of pulmonary hypertension (PH) is considered, by several authors, determinant of clinical outcome. Plasmatic N-terminal-pro-B type natriuretic peptide (NT-proBNP) might be useful in diagnosis and management of PH in newborns, although its interest in CDH infants remains to be defined. Early NT-proBNP levels were assessed in CDH infants and correlated with cardiovascular echocardiographic parameters. ***Patients and Methods:*** 28 newborns, CDH and age-matched controls were enrolled in a prospective study. Clinical condition, NT-proBNP plasmatic levels, echo parameters of PH and biventricular function were assessed at 24 h after delivery as well as survival outcome. ***Results:*** Estimated mean pulmonary pressure and NT-proBNP were significantly higher in CDH than control infants. NT-proBNP significantly correlated with estimated pulmonary artery pressure, right ventricular Tei index, and tricuspid E/A ratio. Additionally, we found that CDH infants with NT-proBNP >11,500 pg/ml experienced a worse prognosis. ***Conclusions:*** We demonstrated that PH is associated with NT-proBNP elevation and diastolic impairment in CDH infants. Early elevations in NT-proBNP levels seem to alert for a subset of CDH infants with worse prognosis.

Introduction

Despite many advances in the management of congenital diaphragmatic hernia (CDH), the outcome of affected infants remains unpredictable and varies from year to year even in experienced teams with predetermined treatment protocols. Its morbidity and mortality is largely dependent of pulmonary hypertension (PH) and hypoplasia [1]. Pulmonary hypoplasia installs during prenatal development [2–4] and limited possibilities exist to attenuate it [5, 6]. PH is likely secondary to pulmonary

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hypoplasia and associated underdeveloped vascular bed, which for some authors is a major determinant of postnatal clinical outcome [7]. For this reason, evaluation of PH severity is considered important for management of CDH infants in several centers [1] and its assessment is crucial to decide upon pulmonary vasodilator therapy as well as to monitor its effects [8, 9]. Furthermore, for these authors the decision for surgical repair should be based on evidence of PH stabilization [10]. Assessment of PH is mainly based on clinical and echocardiographic estimation. However, echocardiographic evaluation is not always available and it is somewhat observer-dependent and technically demanding in CDH infants due to the presence of abdominal organs in thoracic cavity. An easy and reliable method to assess PH remains to be defined. On the other hand, PH could induce cardiac dysfunction due to right ventricular (RV) overload, although this aspect has not been clearly elucidated in CDH infants [11].

B-type natriuretic peptide (BNP) is a hormone of predominantly ventricular origin produced and released in response to increased ventricular wall stress [12, 13]. N-terminal-pro-BNP (NT-proBNP), the amino-terminal portion of the preprohormone, is secreted into the peripheral blood in equimolar portions to BNP, but it has a longer half-life and is easier to measure [13]. In recent years, NT-proBNP has emerged as a very sensitive biochemical marker for ventricular dysfunction in adult heart failure, the plasmatic level of which could be used as a guide for the response to therapy and to predict prognosis [14]. However, in children the knowledge about the significance of plasma levels of NT-proBNP is still limited. In healthy children, studies have shown that NT-proBNP levels are elevated soon after birth reaching its peak at 24 h of life decreasing thereafter up to 4 months and remaining unchanged until the age of 15 [15–19]. NT-proBNP levels are elevated in children with congenital heart disease or cardiomyopathy [20–22]. In infants, it was also demonstrated that NT-proBNP is elevated in symptomatic patent ductus arteriosus in preterms [23, 24] and PH [25]. Additionally, in newborns submitted to cardiac catheterization due to critical pulmonary stenosis or atresia, NT-proBNP correlates with hemodynamic measured RV pressure [26]. However, assessment of plasmatic NT-proBNP and its clinical usefulness in newborns with PH due to CDH remains to be defined.

The aim of this study was to compare echocardiographic parameters of cardiac function and plasmatic NT-proBNP levels, at 24 h after birth, between CDH and healthy infants.

Materials and Methods

Study Subjects

From January 2004 to October 2006, we enrolled in this study term or near-term newborns (≥36 weeks' gestation) admitted to our hospital's neonatal intensive care unit (NICU) or normal newborn nursery. Two groups were defined: (1) a control group that included healthy infants, and (2) a CDH group that included consecutive newborns with left-sided CDH (Bochdalek hernia) without heart defect. Sample size was calculated in order to detect a difference of at least 2 units between groups' means, assuming a standard deviation of 1 unit, with a 95% confidence level (5% α level) and at least 85% power, in an independent samples Student's t test. Thus, we estimated having to recruit at least 20 newborns (10 CDH patients and 10 controls).

Healthy infants were identified from patients that needed blood sampling, at the second day of life, for clinical reasons not related to this study, specifically by suspected sepsis or physiologic jaundice. In these infants, echocardiography was performed immediately before blood was drawn for functional evaluation and exclusion of congenital heart disease. Physiologic jaundice is defined as a benign condition with bilirubin levels <15 mg/day. Infants with confirmed sepsis or newborns with transient tachypnea as well as congenital heart disease, PH or hemodynamic significant ductus arteriosus were excluded from this study.

In CDH newborns, echocardiography was done immediately before blood was drawn that is performed routinely at 24 h of life. Clinical management of CDH infants in our NICU include delayed surgical repair after extensive preoperative stabilization with 'gentle ventilation' and inhaled nitric oxide treatment, if necessary. We could not manage patients with extracorporeal membrane oxygenation (ECMO) since it is not available in our center.

NT-proBNP was measured in the blood drawn in these two groups.

Demographic data from both study groups included postmenstrual age at delivery, gender, birth weight as well as 1- and 5-min Apgar scores. At the moment of blood sampling, data gathered included systolic, mean and diastolic blood pressure, requirement of inotropic support, pulmonary vasodilator therapy as well as of mechanical ventilation, current ventilator settings and blood gases. In CDH infants the oxygenation index and the ventilatory index were calculated. Simultaneously, echocardiographic parameters and plasmatic NT-proBNP levels were recorded. Additionally, we recorded the day of surgery as well as survival taking into account that survivor newborn was defined as alive at discharge from NICU.

The study was approved by the Institutional Review Board of our hospital and informed consent was obtained from the parents of all participants. No infant, neither case nor control, received additional blood collection, other than routine blood sampling, as a consequence of this study, as NT-proBNP usually could be measured in the routine amount of blood sample. Data collected from this study were not used to influence medical decision-making. Management of each infant was left to the criteria of the attending physician, according to the treatment protocol of our NICU. NT-proBNP plasmatic measurement was not achieved in 5 CDH newborns, due to insufficient sample.

Echocardiographic Assessment

The echocardiographic assessment was designed as part of the infants' routine care or additionally to that, at no cost to the patient. All examinations were performed with an echograph Aloka (Tokyo, Japan) using a 5-MHz probe, by the same investigator (M.J.B.). Once echocardiography always preceded blood sampling, the investigator interpreting echocardiography was blinded to NT-proBNP levels, although not blinded for control versus CDH groups.

In infants from control and CDH groups, echocardiography was performed to exclude congenital heart disease and to ascertain the following parameters: (i) RV and left ventricular (LV) systolic and end-diastolic dimensions (M-mode, parasternal ventricular short-axis view); (ii) mitral and tricuspid diastolic dimensions (2D, 4-chamber view); (iii) dimensions of the right and left pulmonary arteries (RPA and LPA); (iv) orientation of ventricular septum (existence of bulging to left ventricle); (v) ductus arteriosus and foramen oval patency and shunt direction; (vi) existence and quantification of tricuspid regurgitation as well as RV-right atrium (RA) gradient; (vii) RV outflow acceleration time (OAT); (viii) RV outflow ejection time; (ix) peak flow velocity of A and E wave at mitral and tricuspid valves; (x) time from cessation of tricuspid and mitral inflow to onset of tricuspid and mitral inflow, respectively, in the next cardiac cycle. These allowed the estimation of: (xi) mitral-to-tricuspid ratio; (xii) LPA-to-RPA ratio; (xiii) estimated right ventricular systolic pressure (RVSP) through the formula: RVSP = tricuspid regurgitation gradient + RA pressure, assuming a normal RA pressure of 4 mm Hg [27, 28]; (xiv) estimated mean pulmonary artery pressure (MPAP), through the formula MPAP = 90 – (0.62 × OAT) [29]; (xv) tricuspid and mitral E/A ratios; (xvi) the RV and LV Tei index, as described by Tei et al. [30], assessed as Tei index = (time from cessation of tricuspid or mitral inflow to onset of tricuspid or mitral inflow in the next cardiac cycle – RV or LV outflow ejection time)/RV or LV outflow ejection time; (xvii) RV OAT to RV outflow ejection time, a quantitative predictor of peak PA pressure in infants [31]. Measurements were obtained in 3 consecutive cardiac cycles and averaged to account for respiratory variation.

Taking into account the distinct characteristics of RV and LV, we evaluate ventricular function using different parameters: (i) global function, using the Tei index in RV and LV [32]; (ii) systolic function, with peak pulmonary flow velocity in RV [33] and ejection fraction in LV; (iii) diastolic function, tricuspid and mitral E/A ratio in RV and LV, respectively.

In CDH newborns the echocardiographic window is limited due to the intrathoracic position of abdominal organs and mechanical ventilation. Nevertheless, the echocardiographic protocol was completed in all CDH patients. For that purpose the subcostal window or right-sided parasternal window was sometimes used instead of the classical echo windows.

NT-proBNP Measurement

The plasmatic level of NT-proBNP was evaluated at 24 h of life in every control infant and in 13 of the 18 CDH patients. NT-proBNP levels were measured with a chemiluminescent immunoassay kit (Roche Diagnostics, Portugal) on an Elecsys 2010 analyzer. Venous blood samples were collected in EDTA-containing tubes.

Statistical Analysis

Descriptive statistics were presented as mean and standard deviation as appropriate. Plasma NT-proBNP measurements resembled a log-normal distribution, so natural logarithmic transformation was used to normalize the distribution when indicated. Comparisons between groups were performed using Fisher's exact test for categorical variables. For continuous variables comparisons, we used Student's t test if normality of the distributions could be assumed or non-parametric Mann-Whitney U test if normality could not be assumed. In order to test the normality of the distribution of the continuous variables the one-sample Kolmogorov-Smirnov goodness-of-fit test was applied. Pearson correlation coefficients were calculated to evaluate the relationship between NT-proBNP and estimated pulmonary pressure as well as the echocardiographic parameters of cardiac function. To describe the discriminative power of plasma NT-proBNP measurement concerning mortality outcome, sensitivity and specificity were calculated for various levels of this variable, a receiver operating characteristic (ROC) curve was drawn. Given the small sample size it was not possible to analyze the prognostic value of plasma NT-proBNP measurements using appropriate multivariate modeling methods. For hypothesis testing, a value of $p < 0.05$ was considered significant.

Results

In this study, we evaluated 28 infants, 10 in the control group and 18 in the CDH group. In the CDH group, NT-proBNP was evaluated only in 13 newborns, due to an insufficient amount of blood for the measurement. The demographic data of CDH infants are presented in table 1. Excluding Apgar scores in CDH infants, no significant differences were identified with regards to birth weight, gestational age and gender.

The clinical conditions of CDH newborns, regarding the requirement of inotropic support, pulmonary vasodilator therapy as well as day of surgery and outcome, are summarized in table 2. NT-proBNP levels were significantly higher in CDH than in the control group (fig. 1).

Evaluated echocardiographic parameters in control and CDH groups are presented in table 3. In newborns with CDH, estimated MPAPs were significantly higher when compared to the control group reflecting an increased afterload to the RV. All newborns from CDH groups had some echocardiographic evidence of PH, bulging of interventricular septum and right-to-left shunt at the level of ductus arteriosus and foramen ovale. Morphologically, RV systolic and diastolic dimensions were significantly higher in the CDH group than the control group, whereas LV systolic and diastolic dimensions were significantly lower in the CDH than the control group.

Table 1. Demographic data

	Groups studied		p value
	control group (n = 10)	CDH group (n = 18)	
Birth weight, g	3,236 ± 612	2,954 ± 451	0.175[1]
Gestational age, weeks	38.3 ± 1.3	37.7 ± 1.7	0.430[1]
Male gender	9 (90)	12 (67)	0.364[2]
1-min Apgar, median	9	7	0.003[3]
5-min Apgar, median	10	8	<0.001[3]
Hours of measurement	26 ± 1.2	23 ± 0.9	0.463[1]

CDH = Congenital diaphragmatic hernia. For continuous variables, values are expressed as numbers of infants (%) or means ± SD.

[1] t test for independent samples. [2] Fisher's exact test. [3] Mann-Whitney non-parametric test.

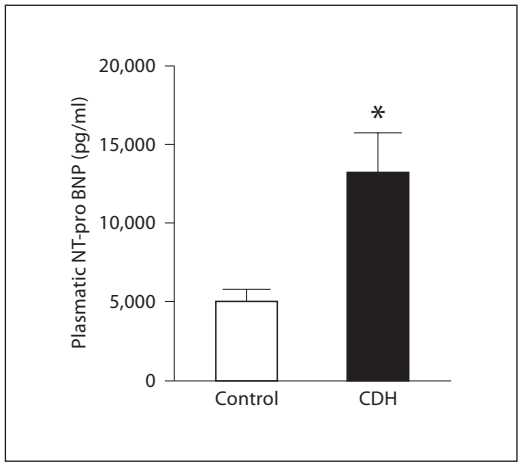

Fig. 1. Plasmatic NT-proBNP level is significantly increased in newborns with CDH compared to control (* $p < 0.05$ vs. control).

Table 2. Clinical data of CDH newborns

Infant	Inotropic support	Vasodilator therapy	Mechanical ventilation	Day of surgery	Day of discharge
1	Yes (dopamine and dobutamine)	Yes (iNO)	SIMV (for 24 days)	D14	D24 (death)
2	No	No	SIMV (for 10 days)	D7	D14 (alive)
3	Yes (dopamine and dobutamine)	No	SIMV (for 2 days)	No	D2 (death)
4	Yes (dopamine and dobutamine)	No	SIMV (for 2 days)	No	D2 (death)
5	No	No	SIMV (for 6 days)	D4	D13 (alive)
6	Yes (dopamine and dobutamine)	No	SIMV (for 12 days)	D4	D18 (alive)
7	No	No	SIMV (for 6 days)	D4	D11 (alive)
8	Yes (dopamine)	No	SIMV (for 7 days)	D4	D24 (alive)
9	Yes (dopamine and dobutamine)	No	SIMV (for 3 days)	D3	D3 (death)
10	No	No	SIMV (for 14 days)	D5	D21 (alive)
11	Yes (dopamine and dobutamine)	Yes (iNO)	HFVO (for 89 days)	D42	D89 (death)
12	Yes (dopamine and dobutamine)	Yes (iNO)	HFVO (for 20 days)	D9	D38 (alive)
13	Yes (dopamine)	No	SIMV (for 7 days)	D3	D17 (alive)
14	Yes (dopamine)	Yes (iNO)	SIMV (for 6 days)	No	D6 (death)
15	Yes (dobutamine)	Yes (iNO)	SIMV (for 35 days)	D14	D46 (alive)
16	Yes (dopamine)	Yes (iNO)	SIMV (for 8 days)	D8	D21 (death)
17	Yes (dopamine)	Yes (iNO)	SIMV (for 11 days)	No	D11 (death)
18	Yes (dopamine)	Yes (iNO)	SIMV (for 13 days)	D4	D13 (death)

iNO = Inhaled nitric oxide; SIMV = synchronous intermittent mandatory ventilation; HFOV = high-frequency oscillatory ventilation.

Additionally, mitral-to-tricuspid and LV-to-RV diastolic M-mode ratios were significantly lower in the CDH than the control group.

Evaluation of global function parameters revealed that the RV Tei index was significantly higher in the CDH than the control group. Regarding systolic function, in comparison to the control group, CDH infants had a slightly depressed RV function as evidenced by a significant decrease in peak pulmonary velocity, whilst the LV systolic function was enhanced as demonstrated by a higher LV ejection fraction. Both RV and LV diastolic parameters (E/A auriculo-ventricular ratios) were signifi-

Table 3. Data of echocardiography in control and CDH infants at 24 h after birth

	Control group	CDH group	p[a]
Pulmonary pressure			
Pulmonary systolic pressure, mm Hg[b]	–	56.0 ± 18.6	
Pulmonary mean pressure, mm Hg[b]	41.6 ± 14.9	64.0 ± 14.9	0.001
Heart dimensions			
Right ventricle			
Tricuspid diameter, mm	9.45 ± 1.00	10.45 ± 2.00	0.055
RV systole M-mode, mm	5.75 ± 2.70	9.40 ± 4.70	0.017
RV diastole M-mode, mm	7.00 ± 2.15	11.30 ± 3.60	0.009
Left ventricle			
Mitral diameter, mm	9.90 ± 1.80	8.15 ± 1.80	0.019
LV systole M-mode, mm	10.40 ± 1.10	7.90 ± 3.90	0.023
LV diastole M-mode, mm	16.00 ± 1.40	14.30 ± 3.50	0.025
Ratios			
Mitral-to-tricuspid	1.03 ± 0.15	0.79 ± 0.15	<0.001
RPA-to-LPA	1.15 ± 0.30	1.10 ± 0.20	0.071
LV-to-RV diastole M-mode	2.35 ± 0.65	1.10 ± 0.40	0.002
Heart function			
Global function			
RV Tei index	0.21 ± 0.05	0.28 ± 0.09	0.002
LV Tei index	0.22 ± 0.17	0.23 ± 0.10	0.674
Systolic function			
Peak pulmonary velocity, m/s	0.80 ± 0.18	0.59 ± 0.12	0.003
LV ejection fraction, %	67 ± 9	80 ± 17	0.02
Diastolic function			
Tricuspid E/A ratio	0.96 ± 0.11	0.80 ± 0.13	<0.001
Mitral E/A ratio	1.00 ± 0.44	0.81 ± 0.13	0.019

Results are presented as median ± interquartile range.

CDH = Congenital diaphragmatic hernia; RPA = right pulmonary artery; LPA = left pulmonary artery; LV = left ventricle; RV = right ventricle.

[a] Mann-Whitney non-parametric test. [b] Both mean and systolic pulmonary pressure were estimated.

cantly lower in the CDH group than the control group, suggesting significant RV and LV diastolic impairment in the CDH group. We found significant correlations between NT-proBNP and estimated pulmonary mean pressure ($r = 0.45$; $p = 0.03$), RV Tei index ($r = -0.50$; $p = 0.02$) and tricuspid E/A ratio ($r = -0.46$; $p = 0.03$).

Comparison between Survivors and Non-Survivors

Nine infants of the CDH group died. A comparison between survivors and non-survivors is presented in table 4. NT-proBNP plasmatic level, at the end of the first day of life, was significantly higher in non-survivor than in survivor newborns. There are no differences between both groups concerning birth weight, gestational age, 1- and 5-min Apgar or mean arterial blood pressure. Regarding pulmonary indices, we detected significant differences between survivor and non-survivor infants. On echo parameters, we identified that estimated mean pulmonary pressures were significantly higher in non-survivor than in survivor CDH infants, whereas pulmonary acceleration to ejection time and tricuspid E/A ratios were significant lower in non-survivor than in survivor CDH infants.

The NT-proBNP level of 11,500 pg/ml was the value with highest specificity and sensitivity to separate survivor and non-survivor CDH infants in ROC curves, in CDH infants. According to the ROC curve, the cutoff NT-proBNP level of 11,500 pg/ml has 100% sensitivity and 67% specificity. Infants with a plasmatic NT-proBNP at 24 h of life >11,500 pg/ml had a significantly lower survival rate than those with plasmatic NT-proBNP at 24 h of life <11,500 pg/ml.

Table 4. Parameters studied at 24 h of life in newborns with CDH according to survival

	Survivors (n = 9)	Non-survivors (n = 9)	p
NT-proBNP, mg/ml	6,230 ± 4,743	22,653 ± 5,653	0.009[2]
log[NT-proBNP] *	8.73 ± 0.87	10.14 ± 0.30	0.003[1]
Demographic data			
Birth weight, g *	3,098 ± 415	2,882 ± 620	0.238[2]
Gestational age, weeks *	38 ± 1.4	37 ± 2.5	0.686[1]
Male gender, n (%)	6 (67)	6 (67)	0.999[3]
1-min Apgar	8 ± 1	7 ± 3	0.21[2]
5-min Apgar	9 ± 1	8 ± 3	0.178[2]
Mean arterial blood pressure, mm Hg *	40 ± 8	41 ± 10	0.449[1]
Pulmonary indices			
PaO_2	80 ± 20	42 ± 16	0.003[2]
$PaCO_2$	46 ± 15	58 ± 16	0.063[2]
FiO_2	0.3 ± 0.6	1.0 ± 0.0	0.002[2]
MAP	6.9 ± 1.9	12.8 ± 1.5	0.003[2]
Oxygenation index	2.2 ± 5.0	28.8 ± 24.26	0.003[2]
Ventilatory index	243 ± 141	879 ± 139	0.003[2]
Echo indices			
Mitral-to-tricuspid ratio	0.82 ± 0.20	0.77 ± 0.07	0.596[2]
LV-to-RV diastole M-mode ratio	1.2 ± 1.0	1.1 ± 0.2	0.400[2]
Pulmonary systolic pressure, mm Hg	42 ± 19	58 ± 19	0.077[2]
Pulmonary mean pressure, mm Hg	53 ± 11	68 ± 0.6	0.009[2]
RV Tei index	0.27 ± 0.05	0.31 ± 0.11	0.411[2]
LV Tei index	0.22 ± 0.09	0.23 ± 0.07	0.999[2]
Peak pulmonary velocity, m/s	0.58 ± 0.17	0.62 ± 011	0.700[2]
Pulmonary acceleration/ejection time	0.33 ± 0.1	0.22 ± 0.03	0.002[2]
Tricuspid E/A ratio	0.85 ± 0.11	0.76 ± 0.29	0.020[2]
Mitral E/A ratio	0.82 ± 0.15	0.79 ± 0.23	0.191[2]

Results are presented as median ± interquartile range unless otherwise indicated. * mean ± standard deviation.

[1] t test. [2] Mann-Whitney non-parametric test. [3] Fisher's exact test.

PaO_2 = Oxygen arterial partial pressure; $PaCO_2$ = carbon dioxide arterial partial pressure; MAP = mean airways pressure; LV = left ventricle; RV = right ventricle.

Discussion

In our study, we demonstrated that PH is associated with diastolic impairment and higher NT-proBNP levels measured at 24 h of life elevation in CDH infants. Early elevations in NT-proBNP levels seem to alert for a subset of CDH infants with worse prognosis.

In recent years, PH has emerged for many authors as a key determinant of outcome in CDH infants [1]. Consequently, PH progressively becomes one of the therapeutic targets in managing these newborns. In fact, in several therapeutic approaches (delayed surgery, high-frequency ventilation, nitric oxide inhalation, ECMO and other pulmonary vasodilators), one of the aims is controlling PH, whereas the decision for surgical repair is based on evidence of PH stabilization [9]. Since accurate evaluation of pulmonary artery pressure with a Swan-Ganz catheter, in newborns, is not achievable, echocardiography is used to assess PH. Nevertheless, echocardiography is not available 24 h a day in all centers dealing with CDH infants and reliable parameters are not easy to quantify, most of them being observer-dependent. In this setting, non-invasive and examiner-independent parameters could be particularly useful to manage CDH infants with PH, but should be available in a short time frame [34].

In this context, we investigated the potential significance of plasmatic NT-proBNP levels in CDH infants. The elevated levels of NT-proBNP that we found in CDH infants should be secondary to PH. Previous studies have demonstrated both in adults [35] and infants [24] that plasmatic NT-proBNP levels are elevated in patients with

PH. Although already demonstrated in adults, the correlation between pulmonary artery pressure and NT-proBNP has not previously been established in infants. In the present study we demonstrated that both in controls and CDH infants the plasmatic NT-proBNP level correlates with estimated mean pulmonary pressure. In CDH infants, invasive measurement of pulmonary pressure and hemodynamic parameters of cardiac function is not feasible. Currently, magnetic resonance imaging is employed in children and adults to evaluate RV, but this is also not reasonable in critical CDH infants.

We decided to measure the NT-proBNP at 24 h of life basically for two reasons: (1) it has been previously demonstrated that NT-proBNP reaches its peak by the end of the first day of life in healthy infants [15], and (2) it is well known that a number of CDH infants do well during the first day of life ('honeymoon period'), but after this period the clinical status of the most severely affected subset of CDH infants deteriorates. For these reasons, the evaluation of NT-proBNP at the end of the first 24 h of life appears appealing, since it could alert for those babies that will require more sophisticated methods to support their life such as ECMO. In fact, we hypothesize that the measurement of NT-proBNP could separate two groups of CDH infants with different prognoses. In our study, plasmatic NT-proBNP >11,500 pg/ml selected those CDH infants that experienced a worse prognosis.

In our study, we performed an exhaustive cardiac function assessment by echocardiography which revealed significant adaptation in biventricular function, both in systole and diastole. The systolic RV function is impaired in CDH infants, probably in relation with higher RV afterload. In CDH infants we found an increase in LV ejection fraction, which could be related with relative underfilling of the LV as well as with end-systolic septal bulging [36]. In fact, it was previously demonstrated that the end-systolic leftward ventricular septal shift in situations with RV pressure overload results in isolated augmentation of systolic shortening in the septal-to-free wall dimensions [36]. Whereas the potential mechanisms for LV filling abnormalities have been pointed out above, impairment of RV diastolic function might be directly related with increased RV afterload. In fact, afterload is a major determinant of diastolic function. Severe afterload elevations slow down the relaxation rate and elevate diastolic filling pressures due to an upward shift of the end-diastolic pressure-volume relation [37, 38]. Additionally, hypoxia and acidosis, reflected as lower Apgar indices observed in CDH newborns, also impair myocardial relaxation and might therefore further contribute to the impairment of both RV and LV diastolic function. The echocardiographic pattern of impaired relaxation (E/A <1) observed in both ventricles of CDH patients in the present study further reinforce our hypothesis. Global RV function evaluated by the Tei index is depressed in CDH infants. This finding is in line with the recent studies of Grignola et al. [39] who demonstrated that the RV Tei index is a sensitive marker of RV dysfunction in the settings of acute PH.

In the present study we documented a significant correlation between NT-proBNP and estimated mean pulmonary pressure as well as some echocardiographic indices of ventricular function (as RV Tei index and tricuspid E/A ratio). The increase of plasmatic NT-proBNP probably reflects ventricular adaptation to pressure overload secondary to PH and could be defined as a non-specific parameter of RV overload. Plasmatic NT-proBNP probably fluctuates according to the clinical condition of CDH newborns, and therapeutic management that modifies ventricular overload could change the level of this biochemical marker. Nevertheless, it will always reflect the actual hemodynamic status of the infants and their response to the treatment.

As previously referred to by several authors, in our study we documented that non-survivor CDH newborns required more aggressive ventilation with impaired blood gas exchange. In fact, some parameters derived from oxygenation status had been suggested as a potential marker of prognosis [40]. The comparison of survivor and non-survivor CDH infants demonstrated that NT-proBNP and estimated pulmonary mean pressure were significantly different in both groups, probably reflecting the increasing severity of the disease. Nevertheless, with the exception of pulmonary acceleration to ejection time and E/A tricuspid ratios, no other parameters of heart function were significantly different in both groups. We believe that this is due to a failure of echocardiography to accurately evaluate heart function and effectively differentiate degrees of severity in impairment of heart dysfunction, instead of lack of increased adjustments in cardiac function related to PH.

In conclusion, we demonstrated that PH in CDH infants induces cardiac function adaptations, which could be evaluated not only by echocardiography but also by plasmatic NT-proBNP. Although this study was carried out in a small number of patients, this biochemical marker could be an accurate indicator of the severity of the clinical condition and PH in CDH newborns. Thus, it seems reasonable to suggest that CDH infants with early

elevations in NT-proBNP should alert for a subset of CDH infants with worse prognosis that should be considered for further study, pulmonary vasodilator therapy or transfer to an ECMO center.

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