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Cardiovascular biomarkers pro-atrial natriuretic peptide and pro-endothelin-1 to monitor ductus arteriosus evolution in very preterm infants

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ABSTRACT

Background: The diagnostic and prognostic appraisal of patent ductus arteriosus (PDA) in preterm infants is still debatable.
Aims: To compare plasma cardiovascular biomarkers with echocardiographic indices alongside ductus arteriosus (DA) evolution in very preterm infants within the first week of life.
Methods: Mid-regional pro-atrial natriuretic peptide (MR-proANP) and C-terminal pro-endothelin-1 (CT-proET-1) levels were prospectively measured on the second and sixth days of life (DOL) in 52 preterm infants born before 32 weeks of gestation. Echocardiographic indices to define DA patency and significance were simultaneously obtained. Logistic regression and receiver operating characteristics (ROC) analyses were used to assess and quantify the biomarkers' diagnostic capacities.
Results: Thirty infants exhibited PDA on DOL 2; in 21 of these infants, DA was characterized as hemodynamically significant. Treatment failure after a first course of indomethacin was noted in 8 infants (DOL 6), whereas 7 participants underwent later surgical ligation. The diagnostic accuracy of cardiovascular biomarkers was moderate on DOL 2 but high on DOL 6. PDA was the only significant predictor of MR-proANP levels on DOL 6, independent of the effect of clinical confounders (regression coefficient 0.426, R^2 0.60). Infants with MR-proANP ≥850 pmol/l on DOL 2 had 3.9-fold higher risk (95% CI 1.01 to 14.5) of being diagnosed with significant DA, whereas infants with MR-proANP ≥700 pmol/l on DOL 6 had 7.1-fold higher risk (1.9 to 27.2) for pharmaceutical treatment failure.
Conclusion: The cardiovascular plasma biomarker MR-proANP is a promising candidate for monitoring PDA evolution in very preterm infants.

1. Introduction

Patent ductus arteriosus (PDA) is a common bystander of prematurity that, if persistent, may lead to important hemodynamic consequences and may predispose to adverse neonatal outcomes [1]. However, in clinical practice, there is much uncertainty concerning the diagnosis and optimal therapeutic approach of "significant" PDA [1–3]. Although echocardiography is the reference standard to assess ductus arteriosus (DA) patency, stringent criteria to define its hemodynamic significance and/or to guide the decision to treat have not been established [1,2]. Moreover, echocardiography is not always readily available in neonatal intensive care unit (NICU) settings, and a single ultrasound examination can provide only temporary information on the constantly changing hemodynamic status during the transitional period, especially in the presence of a PDA [2]. Based on this premise, the potential role of blood biomarkers in assessing the response to PDA-related hemodynamic stress has been recognized [2,4].

Natriuretic peptides are rapidly acting cardiac hormones released in response to myocardial stretch due to volume and/or pressure overload

Abbreviations: BNP, B-type natriuretic peptide; CT-proET-1, C-terminal pro-endothelin; DA, ductus arteriosus; DOL, day of life; LA/Ao, left atrial to aortic root diameter ratio; LR, likelihood ratio; MR-proANP, mid-regional pro atrial natriuretic peptide; NICU, neonatal intensive care unit; PDA, patent ductus arteriosus.

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[5,6]. Although most of the available evidence on their diagnostic value is derived from studies that used B-type natriuretic peptide (BNP) as a diagnostic marker [7–11], atrial natriuretic peptide (ANP) has also been shown to accurately reflect the PDA-related hemodynamic disturbances in preterm infants [12,13].

In contrast to the short half-life of 1–5 min of circulating ANP [14] the prohormone of ANP (proANP) has a 10-fold longer half-life [15]. A novel and reliable immunoluminometric method targeting the mid-regional proANP (MR-proANP), which is secreted in equimolar amounts to ANP, is currently available [16]. Reports from adults with left ventricular dysfunction suggest that MR-proANP levels may even outperform the pro-peptide of BNP (NT-proBNP) in predicting adverse cardiovascular outcomes [17,18]. In preterm neonates, MR-proANP and NT-proBNP levels are closely correlated and increase in parallel with the transition from fetal to postnatal circulation independent of PDA evolution [16].

Endothelin-1 (ET-1) is a potent vasoconstrictor secreted by endothelial cells in response to various stressors, including pulmonary vascular bed overload due to ductus-mediated left-to-right shunt [19]. Although recent evidence suggests a clear relationship between the stable pro-peptide of ET-1 (CT-proET-1) levels and neonatal respiratory morbidity [20], plasma CT-proET-1 levels have been shown to increase in infants with PDA who subsequently required pharmaceutical intervention [16].

This study was undertaken to explore the capacities of MR-proANP and CT-proET-1 to reflect hemodynamic disturbances related to the persistence of PDA in very preterm neonates and to assess their value as complementary diagnostic and prognostic tools to echocardiography.

2. Methods

This prospective cross-sectional study was performed at the University Hospital of Zurich, Switzerland, between August 2011 and April 2012. Infants were eligible to participate if they were born before 32 weeks of gestational age (as determined by the date of the last menstrual period and first trimester fetal ultrasound biometry), provided that the parents gave informed written consent. Newborns with complex congenital malformations, chromosomal aberrations, and congenital heart disease and those who died within the first week after admission were excluded. The institutional review board approved the study protocol (Cantonal Ethics Committee of Zurich; EK:2011-0223).

Blood samples for biomarker determination were drawn on DOL 2 and DOL 6 simultaneously with routine blood sampling. A blood volume of 500 μl (EDTA microtubes) was collected and immediately transferred to the central laboratory for centrifugation and storage at −20 °C until analysis. MR-proANP and CT-proET-1 levels were measured in batches using a fully automated immunofluorescent assay (KRYPTOR, BRAHMS Biomarkers, Thermo Fisher Scientific, Hennigsdorf, Germany). Assay precisions were determined by either repeated serial measurements (n = 10, intraday) or from day to day (n = 10, interday) at a given concentration with the following results: For MR-proANP: intraday precision was 0.6% at 93.1 pmol/L and 0.7% at 467.1 pmol/L; day to day precision was 2.7% at 94.9 pmol/l and 1.8% at 476.3 pmol/l. For CT-proET-1: intraday precision was 1.6% at 229.9 pmol/L; day to day precision was 4.1% at 226.3 pmol/L. Echocardiographic examinations were performed at the same time point as blood sampling (±1 h) by one experienced investigator (RA) blinded to the blood biomarker results. An Acuson Sequoia ultrasound machine was used for echocardiography (Siemens Medical Solutions, Mountain View, CA, USA). DA patency, the direction and volume of the shunt, and the hemodynamic impacts on the pulmonary and systemic circulation were determined using a standardized approach [2]. Significant PDA was defined according to the following echocardiographic criteria [1]: narrowest ductal diameter ≥ 1.4 mm per kg body weight and/or left atrial to aortic root diameter ratio (LA/Ao) ≥ 1.4 and/or diastolic retrograde flow in the postductal descending aorta. The intra-observer variability for ductal diameter was 20%. Data regarding maternal, pregnancy, and perinatal characteristics and information regarding short- and long-term neonatal morbidities were collected from the medical files and recorded in an electronic database.

Infants with significant PDA at DOL 2 were treated with indomethacin (low-dose regimen: six doses of 0.1 mg/kg per dose with 24-hour intervals), which was commenced after study blood sampling at DOL 2. According to our institution's in-house policy, all newborns with birth weights of ≤1250 g and any PDA at DOL 2 also received indomethacin prophylactically (same dosing regimen as above). Infants in whom DA remained patent despite indomethacin administration received a second course of indomethacin (three doses of 0.2 mg/kg per dose with 12-hour intervals). If a second course of indomethacin also failed and significant PDA was maintained or developed according to above mentioned criteria [1] or clinical deteriorations attributable to PDA were noted, e.g. respiratory step back, surgical ligation was performed. Both attending neonatologists and researchers were blinded to the biomarker levels until data analysis.

Continuous variables are expressed as medians with interquartile ranges and were compared using the Mann–Whitney *U* test. Spearman's rank order correlation was used to explore the relationship between biomarkers and echocardiographic parameters. Simple and multivariable linear regression analysis was applied to explore the effects of patient characteristics, including the need for mechanical ventilation (used as a surrogate for the severity of respiratory disease) on the logarithmic-transformed MR-proANP and CT-proET-1 levels. The discriminatory capacities of the biomarkers were assessed by ROC curve analysis using PDA significance (DOL 2), PDA persistence (DOL 6), and pharmaceutical treatment failure as outcomes of interest. A posterior statistical power analysis revealed that the number of cases included was adequate to evaluate MR-proANP differences on DOL 2 and DOL 6 for ductus persistence, significance and indomethacin treatment failure, at the 0.05 significance level in order to achieve a >85% power in all instances. MR-proANP and CT-proET-1 values with negative likelihood ratios <0.2 were defined as low-risk cutoffs. Two-dimensional classification plots were used to explore the trend of the biomarkers from DOL 2 and DOL 6. All analyses were performed using IBM SPSS software

Table 1
Characteristics of the study population (N = 52).

Maternal, pregnancy and perinatal characteristics	
Age, years	34 (30–38)
Antenatal steroids (complete)	40 (76.9)
Preeclampsia	9 (17.3)
Delivery by cesarean section	44 (84.6)
Neonatal characteristics	
Gestational age, weeks	29.2 (26.9–31.3)
Birth weight, g	1160 (910–1600)
Cord blood pH	7.35 (7.32–7.39)
Apgar score at 5 min	6 (4–8)
Apgar score at 10 min	8 (7–9)
Male gender	24 (46.2)
Postnatal morbidities	
Respiratory distress syndrome	49 (94.2)
Mechanical ventilation	26 (50.0)
Sepsis (positive blood culture)[a]	12 (23.0)
Necrotizing enterocolitis (>IIa)	2 (3.8)
Retinopathy of prematurity (>I)	6 (11.5)
Bronchopulmonary dysplasia (any severity)	21 (40.4)
DA-related characteristics	
Patent DA on day 2	30 (57.7)
Significant DA[b] on day 2	21 (40.4)
Patent DA on day 6	8 (15.4)
Treatment with indomethacin	23 (44.2)
2nd course of indomethacin	8 (15.4)
Surgical ligation	7 (13.5)

Values are expressed as median (interquartile range) or n (%) as appropriate.
[a] Median of sepsis onset was on DOL 11 (interquartile range 8–29), in one infant it was on DOL 2 and in one on DOL 5.
[b] PDA with narrowest diameter ≥1.4 mm/kg or/and LA/Ao ratio ≥1.4.