Research Article

Prognostic Role of Biomarkers for Pulmonary Arterial Hypertension Associated with Bronchopulmonary Dysplasia in Extremely Premature Infants

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To explore the association of the biochemical markers after birth with BPD-PAH, factors independently predicting BPD-PAH risk were identified by multivariate logistic regression. Cut off values were determined by plotting receiver-operator curve (ROC), for the sake of dichotomizing continuous variables that showed independent relation with BPD-PAH risk. The results show that uric acid (UC) and blood urea nitrogen (BUN) contents markedly increased among infants experiencing BPD-PAH in comparison with those without BPD-PAH (11.6 vs. 9.7 mmol/L, \( P = 0.006 \) and 482.0 vs. 249.0 \( \mu \)mol/L, \( P < 0.001 \), separately). As shown by multivariate logistic regression, serum BUN levels (\( OR = 1.143 \)) and uric acid levels (\( OR = 1.034 \)) were important risk factors for BPD-PAH. Through a lot of experiments, the effectiveness and the advanced nature of the framework proposed in this paper are proved effectively. The framework proposed in this paper can provide some reference and thinking for follow-up research.

1. Introduction

Pulmonary arterial hypertension (PAH) related to bronchopulmonary dysplasia (BPD-PAH) in infants is fatal, which has affected more and more premature infants. Generally speaking, right heart catheterisation is the gold standard to diagnose PAH. Echocardiography has also been extensively adopted in screening; however, pulmonologists probably do not have the expertise and need to seek the help of an ultrasonographer every time. As a result, it is necessary to identify biomarkers as the first-line approaches for screening. Many PAH-related biomarkers are identified, like troponin-T and NT-pro brain natriuretic peptide whose measurable contents predict the increase PAH mortality [1]. Although the elevated uric acid (UC) levels can be detected in PAH, the study population has been mostly limited to adults and children. Furthermore, it is not yet clear whether the elevated levels are associated with BPD-PAH in extreme infants. This work focused on investigating the association of first biochemical markers after birth with BPD-PAH occurrence among the extremely premature infant (EPI) population.

2. Materials and Methods

2.1. Patients. We carried out the present retrospective study at the neonatal intensive care unit (NICU) of Shenzhen Maternal and Child Health Hospital, Guangdong Province, China, with the approval of the hospital Institutional Medical Ethics Committee (SFYLS [2019] No. 119). It was unnecessary to obtain informed consent due to the retrospective nature. From January 2017 to December 2018, All EPIs...
(<28-week gestational age and birth weight (BW) <1000 g) were reviewed in this work. Neonates with major congenital abnormalities or those died before BPD was diagnosed were eliminated from this work.

2.2. Clinical Variable Definition. This work defined extreme prematurity as <28-week gestational age or BW <1000 g. The diagnosis of BPD was made upon the necessity of oxygen supplementation at discharge or 36 weeks postmenstrual age (PMA) [2, 3]. The diagnosis of PAH was made upon the presence of tricuspid regurgitation, and the systolic pulmonary arterial pressure was predicted to be over one half of systemic arterial pressure (SAP, based on Bernoulli equation). When there was no measurable tricuspid regurgitation, the diagnosis of PAH was made in the presence of interventricular septum (IVS) end-systolic flattening, regardless of dilatation of right ventricle (RV). Moderate PAH was diagnosed in patients with type I–II or II IVS or if RV pressure was predicted to be 50%-75% of SAP, whereas severe PAH was diagnosed in patients with type II-III or III IVS or if RV pressure was predicted to be 75% of SAP [4].

2.3. Data Collection. Venous blood samples were analyzed for biochemical markers routinely (UniCel DxC 800 Synchron (Beckman Coulter, Georgia)) at hospital’s CME accredited laboratory within the first week of life. Infants’ clinical data were retrieved from the electronic medical record.

2.4. Statistical Analysis. Biochemical markers were presented in a form of median (IQR), while nonparametric test was adopted for analysis. Fisher’s exact and chi-square tests were performed for comparing categorical data accordingly. Factors independently predicting BPD-PAH were identified by multivariate logistic regression. Meanwhile, we calculated odds ratios (ORs) together with relevant 95% confidence intervals (CIs) by logistic regression. Thereafter, cut off values were determined based on receiver-operating characteristic (ROC) curve for dichotomizing continuous variables that showed independent relation with BPD-PAH occurrence. SPSS26 (IBM Corporation, NY) was adopted for statistical analysis.

3. Results

Altogether, EPIs were admitted into the NICU from January 2017 to December 2018. Infants referred from other hospitals and those suffering congenital anomalies were eliminated. Consequently, we enrolled 234 infants for final analyses. BPD-PAH was diagnosed in 21 (9.0%) infants (Figure 1).

3.1. Included Infant Clinical Features. Median gestational age was 25.3 weeks (range: 23.6–27.6 weeks), and median birth weight was 836 g (range: 390–1430 g). Table 1 displays infant clinical features. As revealed by univariable analysis, BPD-PAH group showed an increased conception rate due to early-onset sepsis (57. 1% vs. 19.7%), PDA (76.2% vs. 39.4%), and SGA (23.8% vs. 4.2%, Table 1). Besides, BPD-PAH infants showed decreased gestational age (26.0 vs. 26.5 weeks) and BW (730 vs. 900 g, Table 1).

In comparison of biochemical markers according to BPD-PAH, Table 2 compares first biochemical markers within 7 days after birth between BPD-PAH and non-BPD-PAH infants. UC and blood urea nitrogen (BUN) levels markedly increased among BPD-PAH infants relative to non-BPD-PAH infants (482.0 and 249.0 μmol/L, \( P < 0.001; 11.6 \) and 9.7 mmol/L, \( P = 0.006, \) separately, Table 2).

3.2. Factors Independently Predicting the Risk of BPD-PAH. We incorporated all confounders into multivariate regression. As a result, BPD-PAH susceptibility showed independent relation with BW, early-onset neonatal sepsis, PDA, SGA, BUN, and UC level (see Table 3).

3.3. Cut of Values Determined for UC and BUN and Verification. ROC curves were plotted for determining those cut off values for UC and BUN contents detected 7 days postbirth to assess BPD-PAH susceptibility. In our study population, the area under curve (AUC) values of ROC of uric acid and BUN were 0.957 and 0.682, respectively. Serum uric acid values higher than 397.0 μmol/L within 7 days after birth could detect BPD-PAH with a sensitivity of 85.7% and specificity of 92.0%; serum BUN values higher than 9.9 mmol/L within 7 days after birth could detect BPD-PAH, and the specificity and sensitivity were 80.4% and 90.5%, separately (Table 4).

4. Discussion

As mentioned above, some factors were verified to be related to BPD-PAH occurrence, such as LBW, PDA, and SGA [5, 6]. In fetuses and newborns, the growth of pulmonary blood vessels depends on endothelial cells as well as diverse cytokines and growth factors, with vascular endothelial growth factors (VEGFs) being the critical ones [7–9]. The growth of blood vessel is triggered via the vascular endothelial cells, which form connections and rearrange cellular in the processes of anastomosis, sprouting, vascular network functional remodeling, and lumen formation [10]. The stiffness and thickness of pulmonary arteries are identified among premature infants, which suggests the involvement of vascular impairment in the BPD-PAH etiology [11, 12].

Additionally, early-onset sepsis has been discovered to predict an increased BPD-PAH risk. In recent years, Pan and colleagues discovered that activation of inflammasomes and inflammatory cytokines, which inhibited surfactant level, were the possible mechanism that explained how intrauterine infection affected pulmonary development [13]. Colacco et al. also found that every septic episode predicted the 1.26-time increased likelihood of PAH [14].

In our study population, there were different some initial postnatal biochemical markers between EPIs with BPD-PAH and those with no BPD-PAH. As a result, BUN content postbirth (>9.9 mmol/L) independently predicted BPD-PAH occurrence. The relation of increased BUN levels with a higher BPD susceptibility was interpreted experimentally. Arginine is a urea substrate and a nitric oxide (NO) precursor, which is a critical small molecule related to pulmonary diseases in newborns such as PAH and BPD [15].
Extremely premature infants admitted in NICU ($n=281$)

Excluded ($n=30$) infants referred from other hospitals

Extremely premature infants ($n=251$)

Excluded ($n=17$) Infants died before diagnosis of BPD
Infants with congenital abnormalities $3$

Extremely premature infants were analysed ($n=234$) infants with BPD-PDH ($n=21$)

**Figure 1:** Infant selection flowchart.

| Variables                              | Infants with no BPD-PAH ($n=213$) | Infants suffering BPD-PAH ($n=21$) | $\chi^2/z$ | $P$   |
|----------------------------------------|-----------------------------------|------------------------------------|------------|-------|
| *Gestational age (Wks)                 | 26.5 (25.4, 27.2)                 | 26.0 (25.0, 26.4)                  | -1.965     | 0.049 |
| *BW (g)                                | 900 (750, 1030)                   | 730 (620, 885)                     | -3.072     | 0.002 |
| Gender (male)                          | 120 (56.3%)                       | 10 (47.6%)                         | 0.589      | 0.443 |
| Antenatal steroid treatment            | 151 (70.9%)                       | 18 (85.7%)                         | 2.093      | 0.148 |
| Cesarean section delivery              | 84 (25.4%)                        | 6 (28.6%)                          | 0.104      | 0.747 |
| Gestational diabetes mellitus (GDM)    | 17 (8.0%)                         | 3 (14.3%)                          | 0.972      | 0.324 |
| Maternal hypertension n                | 6 (2.8%)                          | 2 (9.5%)                           | 2.604      | 0.107 |
| Conception by ART                     | 48 (22.5%)                        | 6 (14.3%)                          | 0.392      | 0.531 |
| *1-minute Apgar score                  | 8 (5, 9)                          | 6 (5, 8)                           | -1.407     | 0.159 |
| *5-minute Apgar score                  | 10 (9, 10)                        | 10 (8, 10)                         | -1.697     | 0.090 |
| Surfactant treatment                   | 171 (80.3%)                       | 18 (85.7%)                         | 0.363      | 0.847 |
| Mechanical ventilation                | 106 (49.8%)                       | 13 (61.9%)                         | 1.127      | 0.288 |
| PDA                                    | 84 (39.4%)                        | 16 (76.2%)                         | 10.552     | 0.001 |
| SGA                                    | 9 (4.2%)                          | 5 (23.8%)                          | 13.034     | $\leq0.001$ |
| NRDS                                   | 201 (94.4%)                       | 19 (90.5%)                         | 0.514      | 0.473 |
| Early-onset sepsis                     | 42 (19.7%)                        | 12 (57.1%)                         | 15.082     | $\leq0.001$ |
| IVH grade 3 or 4                       | 45 (21.1%)                        | 6 (28.6%)                          | 0.622      | 0.430 |
| ROP                                    | 71 (33.3%)                        | 12 (57.1%)                         | 4.734      | 0.030 |

Values in *median (IQR) or no. (%). BW: birth weight; PPROM: preterm premature rupture of the membranes; GDM: gestational diabetes mellitus; ART: assisted reproductive technology; SGA: small for gestational age; PDA: patent ductus arteriosus; NRDS: neonatal respiratory distress syndrome; ROP: retinopathy of prematurity; IVH: intraventricular hemorrhage.
As discovered by Zheng et al., monocrotaline caused the disrupted urea cycling within PAH animals [16]. Although there are differences between BPD-PAH and adult PAH, some common points are observed between them, like the NO response [17]. The increased BUN levels were related to the decreased conversion of arginine into NO, thus removing NO’s favorable effects on pulmonary arterial pressure and lung growth [18]. Our study supports the hypothesis that arginase inhibits in vitro angiogenesis while markedly increasing PAH susceptibility among infants who have high BUN levels.

According to our results, UC contents are elevated in infants with BPD-PAH. In the case of severe PAH, hyperuricemia may be possibly explained by the excessive production of urate caused by tissue hypoxia. ATP is consumed by tissue ischemia, which promotes xanthine oxidase expression, thus increasing the levels of xanthine, hypoxanthine, and uric acid [19]. UC contents increase among PH cases, which are in indirect proportion to pulmonary vascular resistance. The underlying mechanism remains unclear, but it is probably associated with disrupted UC excretion and low cardiac output due to tissue hypoxia [20].

This study has the major strength of its clinical applicability. Biochemical markers tests are carried out routinely. To sum up, according to our results, serum UC and BUN contents in the first week of life could be used for the assessment of prognosis in BPD-PAH. Nonetheless, one must be cautious when interpreting our findings. In addition, its retrospective nature resulted in a possible inclusion bias, as our cases were the possible BPD candidates. More prospective research is needed for assessing the prognostic significance of these serum biomarkers.

**Data Availability**

All data underlying the results presented in the study are available within the manuscript.

**Conflicts of Interest**

We declare no conflict of interest.

**Authors’ Contributions**

C.C. and B.L equally contribute to the analysis of the data and drafting the manuscript. B.L., C.C., and M.W. collected and analyzed data; B.L. realized the statistical tests; C.Y. participated in writing the manuscript; all authors carefully read and approved the final version for publication.

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