Biological markers in cord blood for prediction of bronchopulmonary dysplasia in premature infants

X.Y. Tian, X.D. Zhang, Q.L. Li, Y. Shen, J. Zheng

Neonatal Department of Tianjin Central Hospital of Obstetrics and Gynecology, Tianjin (China)

Summary

Objective: The current study aims to determine the contents of biological markers in cord blood and to investigate their feasibility as the predictive indices of bronchopulmonary dysplasia (BPD) in premature infants. *Materials and Methods:* Cord blood was collected from 134 premature infants that had birth weight ≤ 1500 g and gestational age (GA) ≤ 32 weeks at the time of birth. The contents of IL-6, IL-6R, Sgp130, and MMP-9 were determined. Infants' clinical data, as well as their mothers' placental pathological data were also collected. Infants with BPD constituted the BPD group, whereas those without BPD comprised the non-BPD (NBPD) group. Differences in the contents of the biological markers between the groups were analyzed to investigate the correlations of these markers with BPD, and then biological markers that can serve as the predictive factors of BPD were defined. *Results:* GA was negatively correlated with BPD. IL-6, IL-6R, and Sgp130 in the BPD group was higher than those in the NBPD group, whereas MMP-9 in the BPD group was lower than that in the NBPD group. IL-6 was positively correlated with BPD and therefore had a predictive effect on BPD. Sgp130 had a collinear correlation with IL-6, which had a predictive effect on BPD as well. When GA was < 30 weeks and IL-6 was > 46.125 pg/ml, the sensitivity, specificity, and area under curve were 1, 0.59, and 0.849, respectively, indicating a good predictive effect on BPD. *Conclusion:* IL-6 and Sgp130 can serve as the independent predictive cytokines of BPD.

Key words: Premature infants; IL-6; IL-6R; Sgp130. MMP-9; Bronchopulmonary dysplasia (BPD).

Introduction

Bronchopulmonary dysplasia (BPD) is a common respiratory disease in premature infants, especially in very premature infants. It is a major reason for increasing incidence and mortality rates of premature infants. Approximately 30% of premature infants with birth weight < 1,500 g suffer from BPD (most are those with birth weight between 501 g and 750 g). In recent years, the incidence of BPD shows an increasing tendency, which is generally presumed to reach 43% among the premature of < 1500 g [1]. In addition, long-term BPD results in nervous system dysplasia and long-term pulmonary dysfunction [2, 3] seriously threaten premature infants' life quality. In China, as neonatal intensive care techniques develop, more and more premature and very low birth weight infants have survived. Consequently, the incidence of BPD is also increasing, which seriously influences the survival rate and life quality of premature infants in this country. Therefore, early prevention and diagnosis of BDP play critical roles in improving premature infants' survival rate and long-term prognosis.

In the past two decades, great achievements have been obtained from studies on the correlation between prenatal infection/inflammation and newborn respiratory prognosis [4]. Many clinical and animal models have proved that although maternal histological chorioamnionitis (HCA) can promote fetal lung maturity, it increases the risk of premature labor and influences the long-term prognosis of premature lung [5, 6]. Furthermore, another study has shown that changes in bioproteins are literally the form of fetus' responses to intrauterine inflammation [7]. These changes play a part in the pathogenesis of BPD. Therefore, inflammatory mediators and some bioproteins serve as the best choices for the early prediction of premature BPD among various factors. Additionally, cord blood is the most easily obtainable blood sample and its obtaining does not cause harm to the premature.

Based on the aforementioned, cord blood samples were taken in the current study. Biological markers in the samples were determined to investigate their correlations with PBD and then to elucidate their feasibility being the predictive indices of BPD.

Materials and Methods

Subjects

A total of 134 very low birth weight infants born at Tianjin Central Hospital of Gynecology Obstetrics between January 1, 2010 and January 31, 2011 were recruited. Their birth weights were ≤ 1,500 g with gestational age (GA) ≤ 32 weeks. All GAs were estimated within 24 hours after birth using new Ballard scoring, and the estimated ages were taken as standard. Of the 134 infants, 35 diagnosed with BPD comprised the BPD group (one had severe BPD, two had moderate BPD, and the others had mild BPD), and the rest constituted the non-BPD (NBPD) group.

Cord blood collection

Three ml of placental umbilical cord umbilical vein blood was taken after neonatal birth. After clotting, the sample was centrifuged at 3000 r/min for 15 min. The supernatant was collected

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Table 1. — *The diagnostic criteria and grading for BPD.*

Diagnosis: Oxygen-inspiring time ≥ 28 d for premature infants	GA	
	< 32 weeks	≥ 32 weeks
Grading: Evaluation time	36 weeks of corrected GA	Postnatal 56 d
Mild BPD	Without oxygen use or discharged from hospital	
Moderate BPD	FiO_2 < 0.30 treatment	
Severe BPD	FiO_2 ≥ 0.30 and/or continuous positive airway pressure or ventilator treatment	

Table 2. — *Comparisons of the general data between groups.*

Group	Cases	GA (weeks) (median; range)	Birth weight (g) (median; range)	Gender	
				Males	Females
BPD	35	29 (26—29)	960 (600—1250)	22	13
NBPDG	99	30 (27—32)	1250 (940—1480)	43	56
Z or χ^2		- 7.605	- 7.118	4,327	
p value		0.000	0.000	0.038	

Table 3. — *Comparisons of the cytokines between groups.*

Group	Cases	MMP-9 (ng/ml) (median; range)	IL-6 (pg/ml) (median; range)	IL-6R (pg/ml) (median; range)	Sgp130 (ng/ml) (median; range)
BPD	35	17.750 (10.250—283.000)	47.000 (0.700—179.750)	29.500 (13.250—191.750)	47.000 (13.750—227.500)
NBPD	99	12.250 (10.000—179.500)	1.500 (0.250—79.850)	14.500 (12.500—118.000)	17.000 (14.000—110.500)
Z		-1.569	-3.323	-3.536	-2.154
p value		0.117	0.001	0.000	0.031

and immediately placed at - 80 °C for detection. All samples were disposed in case that repeated freezing and thawing would influence experimental results.

Placenta collection

A total of 112 fetal placentas were selected and fixed in 10% formalin. They were instantly sent for pathological tests to determine whether HCA existed or not. HCA was defined by the existence of one to three polymorphonuclear leukocytes.

Clinical data and diagnostic criteria

Clinical data of GA, birth weight, sex, respiratory distress syndrome (RDS), ventilator treatment, and BPD were collected.

BPD was defined according to the new definition of BPD given by the BPD reference co-held by the United States National Institute of Child Health and Human Development (NICHD), the United States National Heart, Lung, and Blood Institute, and the Rare Disease Committee; patients' conditions were graded according to severity [8, 9]. The grading results are summarized in Table 1.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed including IL-6, IL-6R, sgp130, and MMP-9 kits and an automatic analyzer.

Statistical analysis

Data were analyzed using the SPSS17.0 software. A normal distribution test was carried out for all variables. Data that did not satisfy normal distribution were evaluated using the rank test and presented as median + range. Multiple factor regression analysis was performed using Logistic.

Results

Treatment comparison

As shown in Table 2, the GAs and birth weights were not in normal distribution, according to normal distribution tests. Correspondingly, rank tests were performed to compare the GAs and birth weights between the groups. The results showed that the GA of the BPD group was significantly younger than that of the NBPD group ($p < 0.05$), and its birth weight was also noticeably lighter than that of the NBPD group ($p < 0.05$). As for gender, males were more than females in number, showing a significant difference ($p < 0.05$).

Cytokine comparison

The expression of IL-6, IL-6R, and sgp130 of the BPD group was significantly higher than those of the NBPD group ($p < 0.05$). Although MMP-9 expression of the BPD group was lower than that of the NBPD group, no significant difference was observed. The results are summarized in Table 3.

The correlation between HCA and BPD

As shown in Table 4, although the incidence rate of HCA in the BPD group was 60% which was higher than that in the NBPD group (54.5%), a Chi-square test did not show a significant difference ($p > 0.05$).

Table 4. — *Correlation between maternal HCA and BPD.*

Group	Cases (%)		χ^2	*p* value
	HCA	Non-HCA		
BPD	21 (60.000)	14 (40.000)	0.312	0.576 > 0.05
NBPD	54 (54.500)	45 (45.500)		

Table 5. — *Correlations of bioactive markers with BPD by multifactor logistic regression analysis.*

Factor	OR	95% CI	*p* value
GA	0.135	0.042-0.436	0.001
Birth weight	0.992	0.987-0.997	0.002
Gender	7.206	1.380-37.636	0.019
IL-6R	0.947	0.912-0.983	0.005
IL-6	1.056	1.019-1.096	0.003

Table 6. — *Correlation of the bioprotein Spg130 with BPD using multifactor logistic regression analysis.*

Factor	OR	95% CI	*p* value
GA	0.383	0.191—0.767	0.007
Birth weight	0.991	0.986—0.996	0.001
Gender	5.525	1.313—23.248	0.020
MMP-9	0.990	0.980—1.000	0.057
Sgp130	1.009	1.000—1.010	0.048

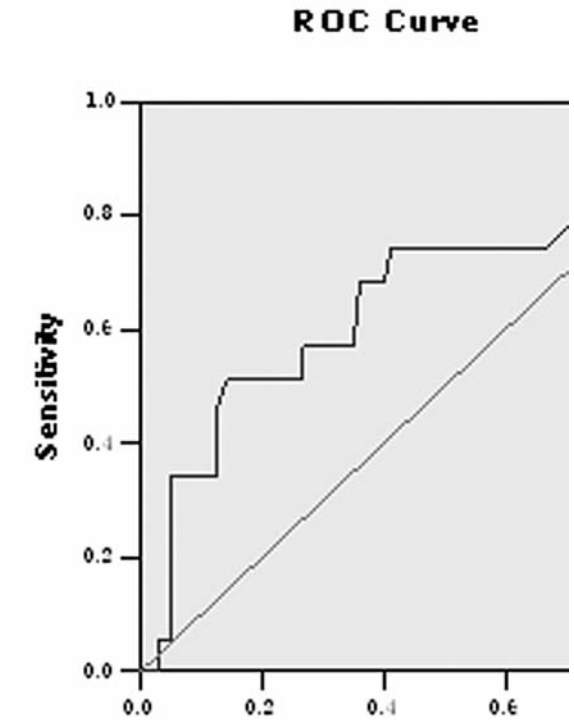

Figure 1. — The predictive values of IL-6 for BPD.

The correlations between bioactive markers and BPD

The Logistic regression analysis showed that GA, birth weight, sex, and IL-6 had influences on BPD: GA, birth weight, and IL-6R were negatively correlated with BPD (namely that a younger GA and a smaller birth weight indicate a higher risk of BPD; both $p < 0.05$) (Table 5), whereas IL-6 was positively correlated with BPD (namely that higher IL-6 expression indicates a higher risk of BPD; $p < 0.05$); males were more [rpme to BPD than females. Additionally, Sgp130 showed collinearity with IL-6 and therefore had a predicative value for BPD (Table 6).

Receiver operating characteristic (ROC) curves

Based on ROC curves, the following can be presumed: IL-6 at 46.125 pg/mL has the highest diagnostic value with sensitivity of 0.51 and specificity of 0.86 (Figure 1); Sgp130 at 47.125 ng/ml has the highest diagnostic value with sensitivity of 0.51 and specificity of 0.82 (Figure 2); and GA < 28.5 weeks and IL-6 > 46.125 pg/ml have sensitivity of 1, specificity of 0.59, and area under the curve of 0.849, which has an good predictive value for BPD (Figure 3).

Discussion

BPD is a common respiratory disease in premature infants, especially in very premature infants. It is a major cause for increases in disease incidence and mortality rates of premature infants. Numerous causes for BPD include external causes such as HCA and postnatal infection, iatrogenic causes such as mechanical ventilation-induced barotraumas and active oxygen free radical injury caused by long time oxygen and high-pressure oxygen, as well as internal host responses [10-12]. More than often, premature infants are exposed to adverse factors such as mechanical ventilation, high-pressure oxygen, barotraumas, and infection; these factors trigger the cascade reactions of inflammatory factors that further exacerbate airway, pneumoangiogram, and interstitial injuries to lead to pulmonary injury [13]. Therefore, inflammatory reaction is a key link in the development of BPD.

HCA is commonly used to describe an inflammatory status of intrauterine tissue, including fetal-maternal mixed tissue (the deciduas and chorial space) inflammation or fetal origin tissue (chorio-amnion, amniotic fluid, and the umbilical core) inflammation [14]. It is often accompanied with the evidence of the invasion of pathogenic bacteria into normal tissues [15, 16]. HCA reduces the incidence rate of RDS in infants with birth weight < 2,000 g but increases the incidence of chronic pulmonary disease [17]. This finding indicates that HCA promotes pulmonary maturation by stimulating adrenal function to promote cortisol secretion on the one hand [18]; on the other, intrauterine infection-induced fetal inflammation significantly disturbs the normal development of the pulmonary structure [19]. Despite these findings, the correlation of HCA with BPD remains controversial. In the present study, although the in-

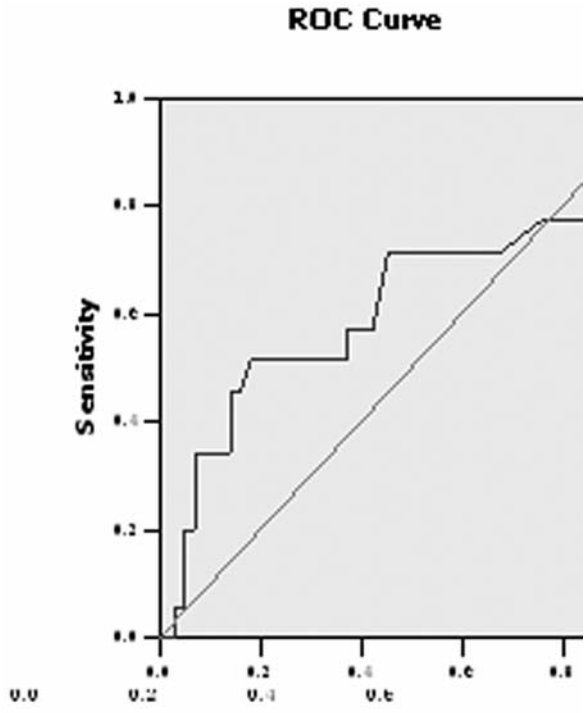

Figure 2. — The predictive values of Sgp130 for BPD.

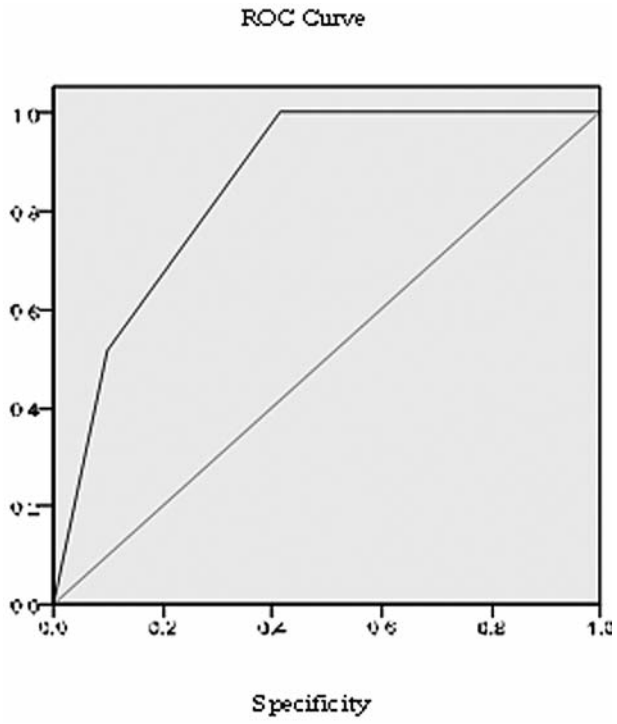

Figure 3. — The predictive values of gestational age < 28.5 weeks and IL-6 > 46.125 pg/ml for BPD.

cidence rate of HCA in the BPD group (60%) was higher than that in the NBPD group (54.5%), no significant difference was observed (no correlation between HCA and BPD is suggested). Presumably, this phenomenon is attributed to the prenatal hormonal therapy performed before delivery at the present hospital, as HCA does not increase the incidence of BPD under the condition of extensive prenatal hormone application [20-22]. However, the correlation between HCA and BPD remains to be explored.

Cytokines are chemical materials secreted by various types of cells such as blood cells, endothelial and epithelial cells, fibroblasts, and type II alveolar cells. They mediate immune, inflammatory, and blood-producing responses caused by stimulation [10], thereby playing a part in the pathogenesis of BPD. In premature infants, stimulation can give rise to general and pulmonary inflammatory responses. Defects in tissue repair, differentiation, and growth can present changes in biomarker concentration as well as corresponding symptoms and signs in BPD infants [23, 24]. Conventionally, IL-6 is presumed to be a type of acute response and lymphocyte stimulating factor [25]. However, recent study has shown that IL-6 performs a series of actions including congenital immune reaction and immune response stimulation [26, 27]. The transformation between congenital and acquired immunity is the key point of inflammatory reaction; obstruction of the transformation can distort immune responses, which ultimately leads to the occurrence of chronic inflammation [28]. An experiment on in vitro fetal membranes has displayed that the levels of IL-6, IL-8, and TNF-α increase in HCA [8]. Another study indicates that premature infants exposed to pro-inflammatory cytokines or those with increased IL-6 in cord blood at the time of birth are more prone to chronic pulmonary disease and that prenatal fetal inflammatory reaction in infants with chronic pulmonary disease is a trigger factor of BPD [29]. The present study is further full proof that the correlation of increased IL-6 with the development of BPD does exit. Therefore, IL-6 level can serve as one of the predictive factor of BPD: IL-6 > 46.125 pg/ml signifies a high risk of BPD with an odds ratio of 1.056, a 95% confidence interval of 1.019 to 1.096, sensitivity of 0.51, and specificity of 0.86; under the condition of GA < 30 weeks, IL-6 > 46.125 pg/ml has a good predictive effect on BPD with sensitivity of 1 and specificity of 0.59. Although these results are basically consistent to those reported by others, differences in the predictive values of IL-6 do exit. Therefore, to further define the standard values of IL-6, larger samples are still necessary.

In addition, IL-6 can only perform its biologic activity by binding with its receptor. In view of the important role played by IL-6 in various diseases, regulating IL-6 expression by applying IL-6 receptor (IL-6R) has become a possible treatment method for associated diseases. IL-6 performs multiple functions in angiogenesis and vascular

remodeling, and it participates in postnatal angiogenesis by circulating endothelial progenitor cells (EPCs) in blood. IL-6R expressed in EPCs (gp80) and the application of IL-6 can activate the gp80/gp130 pathway in EPCs including the phosphorylation of downstream extracellular signal-regulated kinase 1/2 and signal transduction and transcription activator-3 (STAT3) in EPCs, and anti-IL-6 antibodies or IL-6R can inhibit these influences [30]. In this study, because of the existence of the structural reconstruction of pulmonary alveoli in chronic pulmonary disease, IL-6R was detected to clarify whether it performs an effect on pulmonary disease in premature infants. The result shows that IL-6R is negatively correlated with BPD, namely that the lower an IL-6R level is, the more likely BPD is to occur. However, whether the incidence of BPD can be reduced by activating the activity of IL-6R remains to be explored. Meanwhile, the effect of IL-6 on BPD needs further studies to clarify considering that the action mechanism of the IL-6 and IL-6R signal transduction system underlying chronic inflammatory reaction remains unknown. Sgp130 is a cyclic IL-6 receptor subunit for the signal transduction of multiple kinds of cells and plays an important role in the IL-6 signal system. This study shows that in the premature with GA < 30 weeks, an increased Sgp130 level indicates a higher risk of BPD; Sgp130 at 47.125 ng/ml has the largest diagnostic value with sensitivity of 0.51 and specificity of 0.82. However, according to these results, Sgp130 has relatively low sensitivity. This entails large sample sized clinical experiments in the future.

In addition, to date, study has shown that deficiency of the matrix metalloproteinase MMP-9 aggravates pulmonary alveolus agenesia in genetically modified BPD; the activity of MMP-9 may be basically subject to defense mechanism, protecting lung tissues and inhibiting the damage of inflammation to the lungs [11]. In this study, although the MMP-9 content in the BPD group was lower than that in the NBPD group, no significant difference was observed. Therefore, the correlation between MMP-9 and BPD should be explored in the future.

This study shows that the combined consideration of birth weight, GA, IL-6, and Sgp130 for the prediction of BPD can reach accuracy as high as 88.1%. Furthermore, this study shows that males are more prone to BPD than females. However, due to social factors, some very premature female infants did not receive remedy and were excluded from this study. Therefore, whether there is a gender difference in BPD occurrence necessitates further exploration.

With the development of neonatal rescue techniques, the incidence of chronic pulmonary disease in the premature shows an increasing trend. How to predict the disease as early as possible for more effective prevention and treatment remains an urgent problem to solve. Recent years has witnessed bioactive markers become the hot topic of studies on pulmonary disease. Among various types of blood samples, cord blood is the most easily acquired sample whose acquisition does not harm premature infants, as well as the earliest channel for disease prediction. This study shows that IL-6 and Sgp130 have predictive values for BPD. However, their predictive values obtained in this study are not completely in line with those reported (the results reported by others in themselves are not in consistency with each other), and extensive clinical studies are still needed to define more scientific and accurate values. Nevertheless, the application of cytokines is still promising for predicting BPD.

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Address reprint requests to:
J. ZHENG, M.D.
Neonatal Department,
Tianjin Central Hospital of Obstetrics and Gynecology
Tianjin 300100 (China)
e-mail: junzheng268@163.com