

Predictive values of plasma KL-6 in bronchopulmonary dysplasia in preterm infants

Dilek DİLLİ*, Ahmet ÖZYAZICI, Arzu DURSUN, Serdar BEKEN,
Nurdan FETTAH, Ayşegül ZENCİROĞLU, Nurullah OKUMUŞ

Department of Neonatology, Dr Sami Ulus Maternity and Children Research and Training Hospital, Ankara, Turkey

Received: 13.12.2015 • Accepted/Published Online: 09.10.2016 • Final Version: 18.04.2017

Background/aim: It has been suggested that plasma KL-6 increases in premature infants with bronchopulmonary dysplasia (BPD). We aimed to evaluate the predictive values of KL-6 in BPD.

Materials and methods: The study was performed in preterm neonates with birthweight ≤ 1500 g and gestational age ≤ 32 weeks. Plasma KL-6 levels were measured on postnatal days 1, 7, and 14.

Results: BPD was identified in eight of the 28 study infants. On postnatal days 1 and 7, plasma KL-6 levels were similar in infants with BPD [on day 1: 8.9 (7.5–17.8) U/mL and on day 7: 16.8 (10.5–47.7) U/mL] and without BPD [on day 1: 10.8 (9.4–17.2) U/mL and on day 7: 12.9 (5.8–19.3) U/mL] ($P = 0.38$ and $P = 0.13$, respectively). On day 14, KL-6 levels were significantly higher in infants with BPD [155.2 (15.3–545.6) U/mL] than they were in infants without BPD [7.9 (7.7–15.6) U/mL] ($P = 0.001$). The best predictor was KL-6 levels on postnatal day 14 (area under the ROC curve = 0.88; range 0.75–1.0; $P = 0.002$). At this point, KL-6 level of 59.7 U/mL showed a specificity of 90.0% and negative predictive value of 85.7% for BPD.

Conclusion: High plasma KL-6 levels on postnatal day 14 in premature infants may predict the development of BPD.

Key words: Bronchopulmonary dysplasia, KL-6, predictive values, preterm

1. Introduction

Bronchopulmonary dysplasia (BPD) is a syndrome of respiratory distress caused by chronic lung parenchymal injury, occurring especially in preterm infants (1). BPD remains a serious problem in very low birthweight (VLBW) infants despite the use of antenatal steroids and postnatal surfactant therapy to decrease the incidence and severity of respiratory distress syndrome (RDS) (2,3). BPD has a complex and multifactorial etiology, including oxygen toxicity, preterm delivery, hypoxia/hyperoxia, infection, and inflammation (4).

Many studies have suggested that lung inflammation is a major contributor to the pathogenesis of BPD (5,6). Various parameters of lung inflammation may be used to show lung inflammation. However, most of them such as N-terminal propeptide of type 3 collagen, SP-A, anti-SP-A immune complexes, nitrotyrosine, soluble E-selectin, intercellular adhesion molecule-1, allantoin, and aldehydes are not specific for lung disease or are complicated to measure (6). Krebs von den Lundgen-6 (KL-6) is a mucin-like high-molecular weight glycoprotein that is classified into cluster 9 (MUC1) of lung tumor and differentiation antigens. It is preferentially expressed by alveolar type 2

cells and stimulates lung fibrosis through its action as a fibroblast chemotactic factor (7).

There are many studies suggesting plasma KL-6 is increased in adult patients with various types of interstitial pneumonia, characterized by type 2 alveolar hyperplasia and fibrosis (8–11). KL-6 has also shown to be as a useful marker for increased alveolar vascular permeability associated with lung injury. However, plasma KL-6 levels are not elevated in noninterstitial lung diseases such as bacterial pneumonia, bronchial asthma, and pulmonary emphysema (9). Similar pathological changes seen in interstitial pneumonia in the lung are also predominant in BPD (7,12,13). Plasma KL-6 can be a clinically useful early marker for BPD. However, there are few data on the predictive characteristics of KL-6. Therefore, in the present study, we aimed to evaluate the predictive values of KL-6 in BPD within the first days of life.

2. Materials and methods

2.1. Study population

All eligible preterm infants between May 2014 and April 2015 were prospectively enrolled in this study, specifically neonates with birthweight ≤ 1500 g and gestational age ≤ 32

* Correspondence: dilekdilli2@yahoo.com

weeks, and admitted to the neonatal intensive care unit of Dr Sami Ulus Maternity and Teaching Hospital of Ankara, Turkey. Exclusion criteria were maternal chorioamnionitis, congenital heart disease, major congenital abnormalities, or a documented chromosomal abnormality. The preterm infants with early neonatal sepsis (defined as occurring in the first 3 days of life) were excluded. The infants who died of nonrespiratory causes, who did not survive more than 4 weeks for other obvious reasons or were discharged before reaching a postconceptional age of 36 weeks, or for whom blood samples were not obtained on postnatal days 1, 7, and 14 were also excluded. Data on demographic and perinatal characteristics including sex, birth weight, gestational age, pregnancy-induced hypertension, diabetes, premature rupture of membranes >18 h, antenatal steroid administration, mode of delivery, and Apgar scores were recorded. Also recorded were the following pulmonary data: surfactant administration for RDS, days of mechanical ventilation, and total days of supplemental oxygen. The rates of hemodynamically significant patent ductus arteriosus (PDA), clinical or proven (culture positive) sepsis within 15 days after birth, necrotizing enterocolitis (NEC) (>grade 2), and retinopathy of prematurity (ROP) (\geq stage 3) were noted. Length of hospital stay and discharge data were also noted.

BPD was defined in accordance with the guidelines of the National Institute of Child Health and Human Development/National Heart, Lung, and Blood Institute Workshop (National Institutes of Health consensus definition) for infants born at gestational age <32 weeks, that is, treatment with >21% oxygen for at least 28 days. At 36 weeks' postconceptional age, the infants were classified as mild, moderate, or severe BPD based on the required fraction of inspired oxygen (FiO₂): mild BPD, none; moderate, 21% to 30%; and severe, >30% or positive pressure assistance (14).

All preterm infants received respiratory support as per the European Consensus Guidelines on the Management of Neonatal Respiratory Distress Syndrome in Preterm Infants—2013 Update (15). Exogenous surfactant, poractant alfa (Curosurf) or beractant (Survanta), was given within the first 6 h after birth and two further doses, if necessary. Prophylactic surfactant administration (within 15 min of birth) was given to preterm infants of <26 weeks' gestation. Prophylaxis was also given to those with RDS who required intubation for stabilization in the delivery room. After extubation, the infants were administered nasal continuous positive airway pressure. Mechanical ventilation was used to support neonates with respiratory failure, if required. Crossover treatment, from conventional ventilation to high-frequency oscillatory ventilation, was applied to neonates with refractory respiratory failure. In general, supportive treatment during the study was at the

discretion of the attending neonatologist, in accordance with the unit protocols.

2.2. Blood sampling and plasma KL-6 measurement

We obtained heparinized blood samples of 0.5 mL from the infants by venipuncture on postnatal days 1, 7, and 14 during hospitalization (mostly, we salvaged the residual blood that was obtained for routine examinations). The blood samples were immediately centrifuged at 3000 \times g for 10 min at 4 °C to obtain plasma and then stored at -80 °C. The KL-6 levels in plasma were measured using a quantitative colorimetric sandwich enzyme-linked immunosorbent assay kit (Eastbiopharm Co., Ltd., Hangzhou, China) in accordance with the manufacturer's instructions. Each sample was run in duplicate and the mean concentration was calculated. The sensitivity of the kit was 1.12 U/mL with an assay range of 2–600 U/mL.

2.3. Ethics

The regional ethics committee approved the study protocol (file no: 10/765). Investigations were performed only after parents of the study subjects provided written informed consent.

2.4. Statistical analyses

All statistical analyses were performed using SPSS 15.0 (Chicago, IL, USA). Numerical data are expressed as median \pm standard deviation (SD) or median with interquartile range (IQR), according to the normal distribution tested by Kolmogorov–Smirnov test. Differences in numeric variables were assessed using Student's t test or Mann–Whitney U nonparametric two-tailed test, as appropriate. Fisher's exact test was used for the categorical variables. Correlation between variables was analyzed by Spearman's test. Friedman's test was used for one-way repeated measurements. Logistic regressions were used to assess the influence of demographic and perinatal characteristics on KL-6 levels at different time points. To validate the usefulness of KL-6 in predicting BPD, receiver operating characteristic (ROC) curves were created at different time points and cut-off levels were determined when a significant result was obtained. Values of $P < 0.05$ were considered significant.

3. Results

The mean gestational age and birth weight of all subjects were 28.3 \pm 2.1 weeks and 1143 \pm 184 g, respectively. The male rate was 53.6%. BPD was identified in eight of the 28 infants studied. Then the subjects were classified into two groups: BPD group ($n = 8$) and non-BPD group ($n = 20$). BPD was mild in two infants and moderate or severe in six. The Table shows the characteristics of the study subjects according to the groups. Compared to the infants without BPD, those with BPD had significantly lower mean gestational age ($P = 0.001$) and birth weight ($P = 0.001$), lower incidence of antenatal steroid use ($P =$

Table. Clinical characteristics of the subjects with and without bronchopulmonary dysplasia.

	BPD group (n = 8)	Non-BPD group (n = 20)	P-value
Maternal age (years), mean \pm SD	24.6 \pm 1.7	24 \pm 4.6	0.71
Gestational age (weeks), mean \pm SD	25.8 \pm 1.1	29.3 \pm 1.5	0.001
Birth weight, mean \pm SD	971 \pm 158	1212 \pm 147	0.001
Maternal hypertension, n (%)	2 (25)	0 (0)	0.07
Maternal diabetes, n (%)	2 (25)	0 (0)	0.07
Premature rupture of membranes, n (%)	3 (37.5)	11(55)	0.67
Antenatal steroid, n (%)	3 (37.5)	20 (100)	0.001
Cesarean delivery, n (%)	3 (37.5)	16 (80)	0.06
Apgar score, median (IQR)	7 (7–8)	8 (7–8)	0.94
Mechanical ventilation (d), mean \pm SD	15 (5–25)	6 (5–7)	0.02
Supplemental oxygen (d), mean \pm SD	59 (42–75)	23 (20–25)	0.001
RDS, n (%)	8 (100)	16 (80)	0.29
PDA, n (%)	6 (75)	6 (30)	0.04
Sepsis, n (%)	5 (62.5)	8 (40)	0.41
NEC, n (%)	6 (75)	0	0.001
ROP, n (%)	3 (37.5)	4 (20)	0.37
Length of hospital stay, median (IQR)	67 (52–106)	47 (40–64)	0.02
Mortality, n (%)	0	0	NA

BPD: Bronchopulmonary dysplasia; RDS: Respiratory distress syndrome; PDA: Patent ductus arteriosus; NEC: Necrotizing enterocolitis; ROP: Retinopathy of prematurity, IQR: Interquartile range, NA: Not applicable

0.001), and longer duration of mechanical ventilation ($P = 0.02$), with higher rates of PDA ($P = 0.04$), as well as longer hospitalization ($P = 0.02$). None of the study subjects died.

On postnatal days 1 and 7, plasma KL-6 levels were similar in infants with BPD [on day 1: 8.9 (7.5–17.8) U/mL and on day 7: 16.8 (10.5–47.7) U/mL] and without BPD [on day 1: 10.8 (9.4–17.2) U/mL and on day 7: 12.9 (5.8–19.3) U/mL] ($P = 0.38$ and $P = 0.13$, respectively). However, on postnatal day 14, KL-6 levels were significantly higher in infants with BPD [155.2 (15.3–545.6) U/mL] than they were in infants without BPD [7.9 (7.7–15.6) U/mL] ($P = 0.001$). KL-6 levels were significantly elevated from day 1 to 14 in the BPD group ($P = 0.001$), but not in the non-BPD group ($P = 0.07$) (Figure 1).

According to PDA grouping, on postnatal day 1, KL-6 levels were significantly higher in infants with PDA [10.8 (9.7–20.9) U/mL] than they were in infants without PDA [8.9 (6.8–11.2) U/mL] ($P = 0.01$). However, on postnatal days 7 and 14, plasma KL-6 levels were similar in infants

with PDA [on day 7: 11.3 (3.4–21.1) U/mL and on day 14: 10.3 (4.7–448) U/mL] and without PDA [on day 7: 12.9 (9.6–23.2) U/mL and on day 14: 13.4 (7.7–17.4) U/mL] ($P = 0.24$ and $P = 0.87$, respectively).

In infants with and without NEC, KL-6 levels were similar on day 1. However, in infants with NEC, KL6 levels were significantly higher on days 7 and 14 [on day 7: 19.7 (16.8–56.1) U/mL and on day 14: 350.4 (119.5–545) U/mL] compared to infants without NEC [on day 7: 12.9 (5.8–19.3) U/mL and on day 14: 8.1 (7.7–15.6) U/mL] ($P = 0.02$ and $P < 0.001$, respectively). Similarly, on postnatal day 14, KL-6 levels were significantly higher in infants with sepsis [15.6 (11.5–302.6) U/mL] than they were in infants without sepsis [7.7 (4.7–18.1) U/mL] ($P = 0.007$).

When analyzed at three time points, plasma KL-6 levels were only positively correlated with gestational age on day 1 (on day 1: $r = 0.45$, $P = 0.01$, on day 7: $r = -0.24$, $P = 0.21$, on day 14: $r = -0.36$, $P = 0.05$). We also found that total oxygen exposure time is positively correlated with KL-6

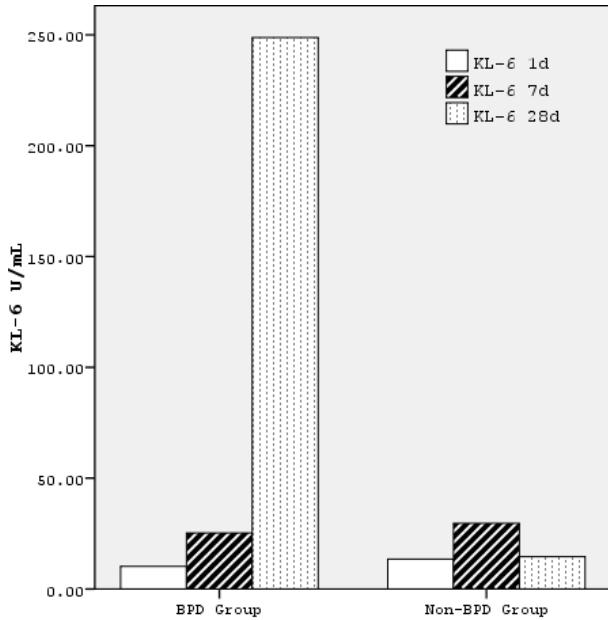


Figure 1. Bar graph showing KL-6 levels on postnatal days 1, 7, and 14 according to the groups.

level on day 14 ($r = 0.46, P = 0.01$). Although plasma KL-6 levels were similar among infants with mild and moderate/severe BPD on days 1 and 7, these levels were higher in infants with moderate/severe BPD on day 14 [on day 1: 10.1 (8.9–14.7), on day 7: 19.7 (16.8–56.1), on day 14: 350 (119–545)] compared to ones with mild BPD [on day 1: 7 (7.1–7.1), on day 7: 8.5 (8–8.5), on day 14: 15 (15.3–15.3)] ($P = 0.07, P = 0.07, P = 0.03$, respectively).

At the three time points the predictive values of KL-6 levels were calculated. The best single predictor was KL-6 levels on day 14 (area under the ROC curve = 0.88; range 0.75–1.0; $P = 0.002$) (Figure 2). At this time point, the cut-off value of KL-6 was determined as 59.7 U/mL. Using this cut-off, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for BPD were 62.5%, 90.0%, 71.4%, and 85.7%, respectively.

4. Discussion

Type 2 pneumocyte hyperplasia with varying degrees of fibrotic change occurs in BPD (16). It is suggested that the pulmonary fibrotic process is caused by injury to alveolar epithelium and basement membrane. During the regenerative process, alveolar type 2 cells proliferate, cover the injured surface, and differentiate into type 1 cells. The regenerating type 2 cells strongly express KL-6 antigen. The serially elevated plasma KL-6 in infants with BPD suggests that these infants had more severe alveolar damage than infants without BPD at least within the first month of life. Ogihara et al. (6) showed significant elevation of plasma KL-6 in the BPD group soon after birth, suggesting KL-6 was a predictor for the early detection of BPD. The present study demonstrated that the serum levels of KL-6 were serially elevated in patients with BPD from postnatal day 1 to 14, while levels did not significantly increase in those without BPD.

In Kurotobi et al.'s study (17), normal levels of KL-6, measured in healthy newborns on postnatal day 1, were reported to be 134 ± 71 U/mL. Uchida et al. (18) measured cord plasma levels of KL-6 in 75 neonates and reported the

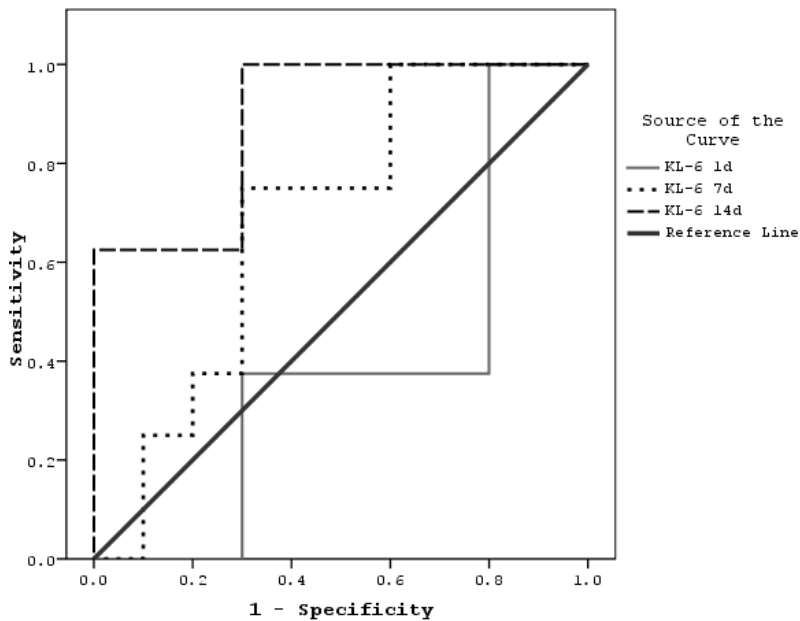


Figure 2. ROC curve analysis of plasma KL-6 levels at three time points for prediction of bronchopulmonary dysplasia.

normal range of values for cord plasma KL-6 as 44.3–148.2 U/mL (median, 73.0 U/mL). In our study the median KL-6 levels were 10.8 (8.8–14.7) U/mL on postnatal day 1, for all subjects. The normal plasma values for KL-6 of these two studies may differ from those of our study because different kits were used.

It was reported that meconium aspiration syndrome (MAS), an acute chemical pneumonitis with consequent disruption of the alveolar–capillary barrier, is also associated with high KL-6 levels (KL-6) (17). However, KL-6 did not increase in infants with RDS or transient tachypnea of the newborn (TTN). It was suggested that KL-6 might have a role as an early indicative marker reflecting the pulmonary remodeling in MAS. Prolonged exposure to hyperoxia may result in the formation of the reactive products of oxygen such as superoxide, and may assist with the progress of oxidative injuries in patients with BPD. Increasing KL-6 levels may reflect the exaggeration of inflammatory processes that result in pulmonary regenerative remodeling or fibrosis, and may indicate the transition from RDS to BPD. In recent years, several researchers have reported that plasma KL-6 levels in preterm infants with BPD were higher than in those without BPD. A systematic review suggested that plasma KL-6 levels could be useful as early markers for predicting BPD in preterm infants (19).

Kurotobi et al. (17) demonstrated that KL-6 was significantly elevated in patients with BPD, but KL-6 levels in those without BPD did not change. Ogihara et al. (6) conducted a prospective study in 135 preterm infants <32 weeks of gestational age. They found that among 42 infants <28 weeks of gestational age, plasma KL-6 levels were significantly higher in those with moderate/severe BPD compared with those with no/mild BPD. They also reported that a plasma level of 199 U/mL at 1 week or 232 U/mL at 2 weeks was an excellent predictor of moderate/severe BPD (PPV of 83% and 80%, respectively). Wang et al. (20) investigated predictive characteristics of KL-6 and Clara cell protein (CC16), another peripheral blood biomarker originating from nonciliated Clara cells, for BPD in preterm infants. They found that serum KL-6 levels higher than 79.26 ng/mL at 14 days postpartum in preterm infants predict the occurrence of BPD. The authors also

stated that CC16 was less predictive than KL-6 at this time point, but KL-6 and CC16 together enhanced the prediction. In their study, the infants with moderate/severe BPD had higher KL-6 levels (on postnatal days 7 and 14), while there were no significant differences in CC16 levels.

In the current study, we followed up 28 newborns <32 weeks of gestational age. Despite the small number of subjects, plasma KL-6 levels were similar among infants with mild and moderate/severe BPD on days 1 and 7; these levels were higher in infants with moderate/severe BPD on day 14. Although the predictive values of KL-6 levels were calculated at three time points (on days 1, 7, and 14), the best single predictor was KL-6 levels (59.7 U/mL) on day 14. Using this cut-off value, PPV and NPV for subsequent BPD were 71.4%, and 85.7%, respectively.

In another study, Yamane et al. (21) showed that infants of earlier gestational age, exposed to longer mechanical ventilation, and with reduced pulmonary function had higher serum KL-6 levels. However, they did not show a significant relationship between serum KL-6 level and pulmonary functions at any postnatal age. They explained that serum KL-6 level was not always a reliable marker of the clinical course of BPD. We observed that total oxygen exposure time was only positively correlated with KL-6 level on day 14.

Our results in the current study may be considered limited by the relatively small sample size, which could have missed small but clinically relevant differences in some of the clinical outcomes. Due to the small number of subjects, we do not have sufficient data to explain the differences between KL-6 levels in preterm infants with and without BPD from the first to the third measurement. The fact that our results are not consistent with the previous research may be caused by several reasons such as sample size, ELISA kit, detection methods, and possible confounding factors.

In conclusion, in this study, we evaluated whether plasma KL-6 might be a predictor of BPD development. Given the high predictive value of the 14th day KL-6 levels in our results, KL-6 seems to be an early marker in BPD. Further studies are required for a better understanding of the biochemical and regulatory roles of KL-6 in preterm infants

References

1. Baraldi E, Carraro S, M. Filippone M. Bronchopulmonary dysplasia: definitions and long-term respiratory outcome. *Early Hum Dev* 2009; 85 (10 Suppl): S1-3.
2. Ambalavanan N, Carlo WA. Ventilatory strategies in the prevention and management of bronchopulmonary dysplasia. *Semin Perinatol* 2006; 30: 192-199.
3. Bhandari V, Finer NN, Ehrenkranz RA, Saha S, Das A, Walsh MC, Engle WA, VanMeurs KP; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Synchronized nasal intermittent positive-pressure ventilation and neonatal outcomes. *Pediatrics* 2009; 124: 517-526.

4. Reyburn B, Martin RJ, Prakash YS, MacFarlane PM. Mechanisms of injury to the preterm lung and airway: implications for long-term pulmonary outcome. *Neonatology* 2012; 101: 345-352.
5. Aghai ZH, Saslow JG, Mody K, Eydelman R, Bhat V, Stahl G, Pyon K, Bhandari V. IFN- γ and IP-10 in tracheal aspirates from premature infants: relationship with bronchopulmonary dysplasia. *Pediatr Pulmonol* 2013; 48: 8-13.
6. Ogihara T, Hirano K, Morinobu T, Kim HS, Ogawa S, Hiroi M, OUE S, Ban R, Hira S, Hasegawa M et al. Plasma KL-6 predicts the development and outcome of bronchopulmonary dysplasia. *Pediatr Res* 2006; 60: 613-618.
7. Kohno N, Akiyama M, Kyoizumi S, Hakoda M, Kobuke K, Yamakido M. Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. *Jpn J Clin Oncol* 1988; 18: 203-216.
8. Yokoyama A, Kohno N, Hamada H, Sakatani M, Ueda E, Kondo K, Hirasawa Y, Hiwada K. Circulating KL-6 predicts the outcome of rapidly progressive idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1998; 158: 1680-1684.
9. Kohno N, Kyoizumi S, Awaya Y, Fukuhara H, Yamakido M, Akiyama M. New serum indicator of interstitial pneumonitis activity. Sialylated carbohydrate antigen KL-6. *Chest* 1989; 96: 68-73.
10. Kashiwabara K, Semba H, Fujii S, Tsumura S, Aoki R. The ratio KL-6 to SLX in serum for prediction of the occurrence of drug-induced interstitial lung disease in lung cancer patients with idiopathic interstitial pneumonias receiving chemotherapy. *Cancer Invest* 2015; 1-6.
11. Hu Y, Wang LS, Jin YP, Du SS, Du YK, He X, Weng D, Zhou Y, Li QH, Shen L, et al. Serum Krebs von den Lungen-6 level as a diagnostic biomarker for interstitial lung disease in Chinese patients. *Clin Respir J* 2015 Jun 16. doi:10.1111/crj.12341.
12. Ishizaka A, Matsuda T, Albertine KH, Koh H, Tasaka S, Hasegawa N, Kohno N, Kotani T, Morisaki H, Takeda J et al. Elevation of KL-6, a lung epithelial cell marker, in plasma and epithelial lining fluid in acute respiratory distress syndrome. *Am J Physiol Lung Cell Mol Physiol* 2004; 286: L1088-1094.
13. Al-Salmi QA, Walter JN, Colasurdo GN, Sockrider MM, Smith EO, Takahashi H, Fan LL. Serum KL-6 and surfactant proteins A and D in pediatric interstitial lung disease. *Chest* 2005; 127: 403-407.
14. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001; 163: 1723-1729.
15. Sweet D, Carnielli V, Greisen G, Hallman M, Ozek E, Plavka R, Saugstad OD, Simeoni U, Speer CP, Halliday HL. European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants—2013 update. European Association of Perinatal Medicine. *Neonatology* 2013; 103: 353-368.
16. Crouch E. Pathobiology of pulmonary fibrosis. *Am J Physiol* 1990; 259: L159-L184.
17. Kurotobi S, Maekawa S, Hara T, Hamana K, Inada N, Kawakami N, Honda A, Matsuoka T, Fujita H, Nagai T. Serum KL-6 level in newborns with meconium aspiration syndrome. *Pediatr Int* 2003; 45: 517-521.
18. Uchida Y, Minowa H, Ebisu R, Nishikubo T, Takahashi Y, Yoshioka A. Normal values for KL-6 in cord venous plasma of neonates. *Pediatr Int* 2007; 49: 167-171.
19. Zhang ZQ, Huang XM, Lu H. Early biomarkers as predictors for bronchopulmonary dysplasia in preterm infants: a systematic review. *Eur J Pediatr* 2014; 173: 15-23.
20. Wang K, Huang X, Lu H, Zhang Z. A comparison of KL-6 and Clara Cell protein as markers for predicting bronchopulmonary dysplasia in preterm infants. *Dis Markers* 2014; 2014: 736536.
21. Yamane M, Yokoyama N. Serum KL-6 level and pulmonary function in preterm infants with chronic lung disease. *Kobe J Med Sci* 2004; 50: 131-140.