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Serum KL-6 Level and Pulmonary Function in Preterm Infants with Chronic Lung Disease

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The purpose of this study was to determine the usefulness of serum KL-6 (sKL-6) level as a clinical marker, in terms of pulmonary function, for infantile chronic lung disease (CLD). The study population comprised 23 infants less than 31 weeks of gestational age admitted to the neonatal intensive care unit of Kobe University Hospital between March 2002 and August 2003. Blood samples were obtained on day 1, 3, 7, and then weekly until 36 weeks. sKL-6 was measured by means of electro-chemiluminescence immunoassay. For evaluation of pulmonary function, static respiratory system compliance (Crs) was measured on the same day. There were nine infants with CLD and 14 without (non-CLD). Peak sKL-6 levels of the non-CLD infants were less than 200 U/ml except for two cases, while those of CLD infants were over 200 U/ml except for one case. Infants of earlier gestational age, exposed to longer mechanical ventilation and with reduced pulmonary function had the higher sKL-6 levels. However, there was no significant relationship between sKL-6 level and Crs at any postnatal age.

INTRODUCTION

KL-6 is a glycoprotein recognized by mouse monoclonal antibody KL-6 and was generated by Kohno et al. in 1988 to identify lung adenocarcinoma associated antigens (4). As a result, the KL-6 antigen was found to be expressed mainly in alveolar epithelial cells type II and in bronchiolar epithelial cells of the healthy human lung, and highly expressed in regenerated alveolar cells in the remodeling region of interstitial pneumonia (6). For this reason, serum KL-6 (sKL-6) level is used as an indicator of disease activity of interstitial pneumonia because KL-6 in damaged pulmonary interstitial tissues leaks into blood capillaries (3, 5, 6, 7, 15). In recent years, several studies have found that sKL-6 level is higher in infants with chronic lung disease (CLD) than in those without (10,11), but there have been no reports only comparing sKL-6 level with pulmonary function. We hypothesized that infants would have both a high sKL-6 level and reduced pulmonary function when the clinical course of CLD worsened. The purpose of this study was thus to determine the usefulness of sKL-6 level as a clinical marker of pulmonary function in CLD.

MATERIALS AND METHODS

Subjects

Premature infants at less than 31weeks of gestational age (GA) with acute respiratory distress after birth, and admitted to the neonatal intensive care unit (NICU) of Kobe University Hospital Perinatal Center between March 2002 and August 2003, were enrolled in

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this study. Infants with any congenital heart disease, multiple malformations, or chromosomal abnormalities and those who could not survive more than 4 weeks for other obvious reasons, were excluded. Informed consents for this protocol were obtained from all parents after birth and before the start of the study.

Blood samples of 0.5ml for measurement of KL-6 levels were obtained on day 1, 3, and 7, and then weekly until 36 weeks. For evaluation of pulmonary function of infants being treated with mechanical ventilation, static respiratory system compliance (Crs) was measured on the same day.

Measurement of KL-6 level

The blood samples were immediately centrifuged at 10,000 rpm for 5 minutes to obtain plasma and stored at -70°C. The sKL-6 level was measured with the Picolumi KL-6 (Eisai Co., Ltd.) by means of electro-chemiluminescence immunoassay (ECRIA). The effective measuring range of the assay was 51 to 10200U/ml, and the coefficient variance was less than 10% for five simultaneous measurements of the same sample. All the samples were analyzed in duplicate.

Evaluation of pulmonary function

Pulmonary function as assessed with the Navigator GM 250 (Newport Medical Instruments, Newport, CA) equipped with a VerFlex flow transducer (Bicore Monitoring Systems, Inc, Irvine, CA). The passive flow-volume method was used to measure static respiratory system compliance (Crs) and for adjustment according to body weight. The measurement of Crs was performed at least five times and three or more repeatable values were averaged.

Data Analysis

We also examined the correlation between sKL-6 levels and Crs to determine whether they were related to each other. sKL-6 levels were compared with Crs according to GA of the patients and whether they had or did not have CLD (non-CLD). The infants were grouped according to various categories: GA, type of CLD, duration of mechanical ventilation and Crs, and the peak sKL-6 levels were assessed for each group. CLD was defined as the need for oxygen supplementation beyond 28 days of age associated with symptoms of persistent respiratory distress and hazy lung fields on chest x-ray films. CLD types were classified from type I to VI, following the classification by the Ministry of Health and Welfare Research Project (9). Statistical analysis was performed using the Mann-Whitney U test, chi-square for independence test and simple regression with StatView 5.0 (SAS Institute Inc.) for Macintosh (Apple Co.), and $p<0.05$ was regarded as significant. The statistically analyzed values are presented as means ± SD.

RESULTS

Twenty-three infants were enrolled in this study, and their characteristics are shown in Table 1. Their mean GA were 25.8±1.6 (23-28) weeks in CLD group and 29.4±0.8 (28-30) weeks in non-CLD ($p<0.0001$; Table 1). Their mean birth weight were 740 ± 184 (494-1018) g in CLD group and 1345±287 (886-1860) g in non-CLD group ($p=0.0002$; Table 1). There were also statistically significant difference in duration of mechanical ventilation and number of the infants who were treated with mechanical ventilation for more than 28 days, who were received postnatal steroids and had O_2 dependency at post conceptional age (PCA) 36 weeks between CLD group and non-CLD group ($p<0.0001$, $p<0.0001$, $p=0.047$ and $p=0.047$ respectively; Table 1). CLD group was consisted of six with type II, two with type III and one with type III' of CLD.

Table 1 Characteristics of infants

	CLD		non-CLD		p value
n	9		14		
Gestational age(weeks)	25.8±1.6	(23-28)	29.4±0.8	(28-30)	p<0.0001
Birth weight (g)	740±184	(494-1018)	1345±287	(886-1860)	p=0.0002
Male : Female	7:2		6:8		n.s.
Antenatal steroids (%)	5	(56%)	6	(43%)	n.s.
Duration of mechanical ventilation (days)	43.0 ±14.3	(24-70)	5.2±3.8	(0-11)	p<0.0001
Mechanical ventilation >28days (%)	8	(89%)	0	(0%)	p<0.0001
Postnatal steroids (%)	3	(33%)	0	(0%)	p=0.047
O_2 dependency at PCA 36 weeks	3	(33%)	0	(0%)	p=0.047

The time courses of sKL-6 level were compared for CLD and non-CLD infants (Fig. 1). Eight of the 9 CLD cases showed elevation of the sKL-6 level to more than 200 U/ml during their clinical course, while twelve of the 14 non-CLD infants had sKL-6 level of less than 200 U/ml. In all of 3 infants in the CLD group received dexamethasone (DEX) therapy because of deterioration of their respiratory condition, sKL-6 levels after DEX therapy were higher than before. The only case in the CLD group with a peak sKL-6 level below 200 U/ml was a male born at GA 25 weeks. He had been treated with mechanical ventilation since birth until 24 days of age followed by treatment with nasal CPAP for 3 weeks. In the non-CLD group, there were two cases with a peak sKL-6 level above 200 U/ml. One was a female born at GA 28 weeks had chorioamnionitis (CAM) with a high IgM value (58mg/dl) at birth and reached her sKL-6 peak level (404 U/ml) at 7 days of age. Another case was a male born at GA 30 weeks without any evidence of prenatal infection. They were treated with mechanical ventilation less than 2 weeks (11 and 5 days, respectively), and then their respiration was stable after extubation until discharge.

For eight cases treated with mechanical ventilation beyond 2 weeks, sKL-6 levels were compared with Crs during two postnatal periods (Fig. 2a, 2b), but no significant relationship between sKL-6 levels and Crs was detected during any postnatal period. During the first 2 to 3 weeks of life, their sKL-6 levels were less than 300 U/ml except for one point, but the corresponding Crs showed a wide distribution. From 4 weeks of age onward, all Crs were above 0.6 ml/cmH_2O/kg, but the corresponding sKL-6 levels showed a wide range of distribution.

The results of the analysis of the relationship between peak sKL-6 levels and categories of all infants are shown in Figure 3. There was the significantly negative correlation between GA and peak sKL-6 levels (peak KL-6=1657-50.5*GA, R^2=0.586, p<0.0001; Fig. 3a). We also could find out the significantly negative correlation between GA and sKL-6 levels at PCA 36 weeks (sKL-6=586-15.0*GA, R^2=0.214, p=0.04; data not shown). CLD infants had a significantly higher sKL-6 levels than non-CLD infants, but there was no significant correlation among the three types of CLD (Fig. 3b). The peak sKL-6 levels of infants treated with mechanical ventilation more than 4 weeks were significantly higher than that of infants treated less than 4 weeks (p=0.002, Fig. 3c).

Fig.1 sKL-6 in CLD and non-CLD infants

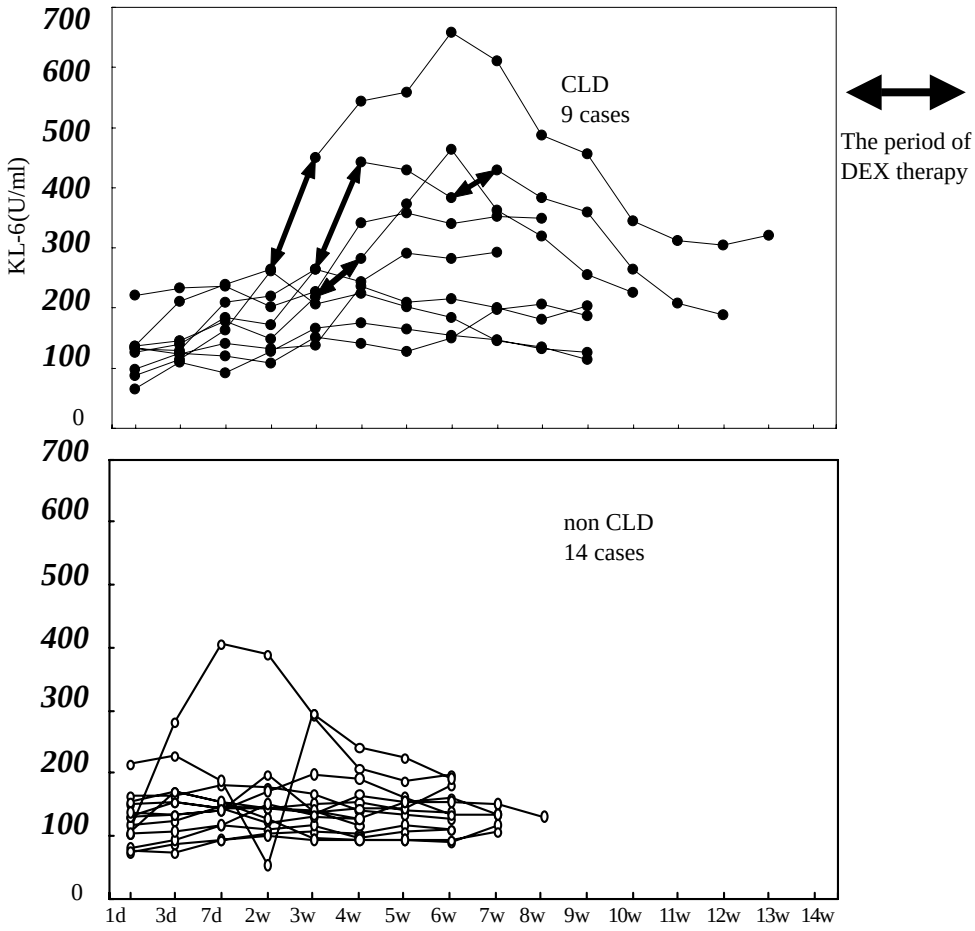

Fig. 1 sKL-6 in CLD and non-CLD infants
Time courses of sKL-6 of all infants. Black circles represent CLD infants, white circles the non-CLD group. Wide arrows mean the period of DEX therapy. In all of 3 infants received DEX, sKL-6 after DEX therapy were higher than before. Eight of 9 CLD infants showed sKL-6 over 200 U/ml depending on their clinical course. The non-CLD infants all had sKL-6 below 200 U/ml except for two cases.

The eight infants treated with mechanical ventilation more than 2 weeks were divided into group of four infants with a lower Crs and the remaining four infants. An infant with a lower Crs was defined as one whose Crs was less than 0.5ml/cmH_2O/kg after 2 weeks of age. A comparison of the peak sKL-6 levels of the infants with the lower Crs and the other infants (Fig. 4) showed that the former had a higher sKL-6 levels than the latter, but there was no statistically significant difference (p=0.08).

Fig.2 Relationship between sKL-6 and Crs in eight ventilated infants

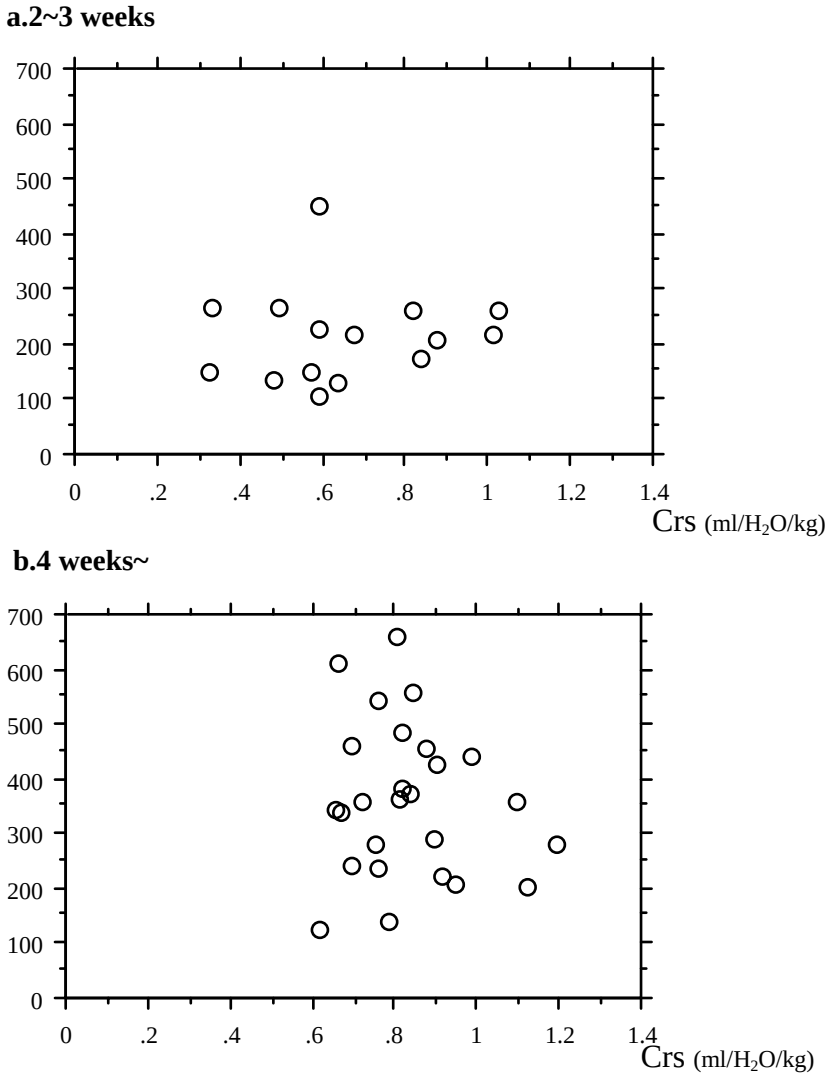

Fig. 2 Relationship between sKL-6 and Crs in eight ventilated infants
sKL-6 and Crs are shown for 2-3 weeks of age (a) and 4 weeks and over (b) for eight ventilated infants over 2 weeks of age. There was no significant relationship between sKL-6 and Crs at any postnatal age. At every time point, sKL-6 shows extensive distribution independent of Crs.

DISCUSSION

In recent years, several groups have reported that sKL-6 levels in preterm infants with CLD were higher than in those without CLD. Although data have been published about sKL-6 levels of CLD and non-CLD infants, the critical level of sKL-6 requiring ventilatory support has not been identified. Manabe et al. previously reported on the usefulness of pulmonary function examination results as extubation criteria for infants with CLD (8). We

Fig.3 Peak sKL-6 and GA, CLD, and duration of mechanical ventilation

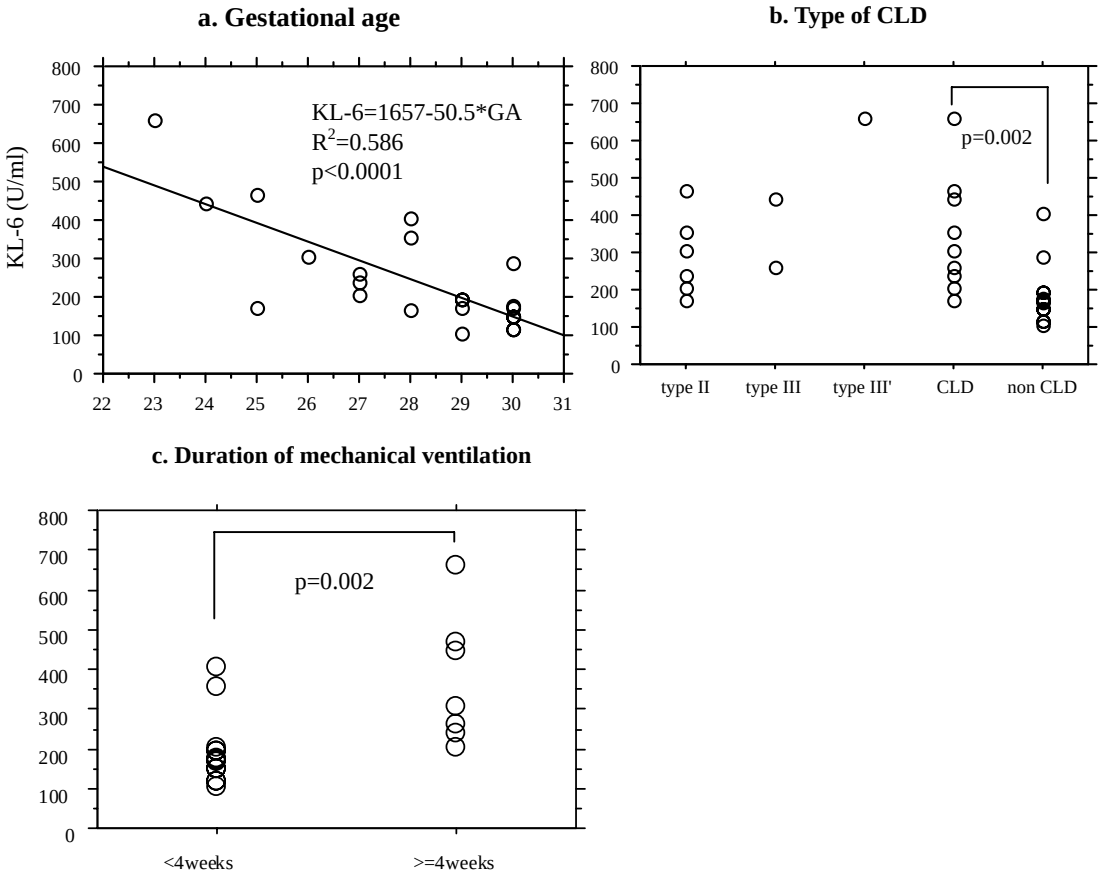

Fig. 3 Peak sKL-6 and GA, CLD and duration of mechanical ventilation
a: Peak sKL-6 according to gestational age; b: Cases classified by type of CLD and absence of CLD; c: Comparison between mechanical ventilation of more than 4 weeks and the others. White circles represent peak values. Infants of a younger gestational age had higher sKL-6 levels. More immature, infants with longer ventilation were also likely to have higher sKL-6 levels.

hypothesized that infants would have both a high sKL-6 level and reduced pulmonary function when the clinical course of CLD worsened. Consequently, this study was designed to investigate the relationship between sKL-6 levels and Crs in mechanically ventilated preterm infants. If any correlation could be established between sKL-6 levels and the corresponding Crs, sKL-6 level should be a very useful marker for the clinical course of CLD because not all NICUs are equipped to measure the pulmonary function of a ventilated infant. Serial measurement of Crs in the CLD group reflected the clinical course of CLD in our study (data not shown). We also examined the relationship between sKL-6 and Crs for eight cases treated with mechanical ventilation for more than 2 weeks during two postnatal periods (Fig. 2) because we speculated that sKL-6 levels at 2 to 3 weeks of age would be very different from those later on due to the extended mechanical ventilation. Although differences were observed in the distribution of sKL-6 levels and the corresponding Crs, we

Fig.4 Peak sKL-6 and Crs in eight ventilated infants over 2 weeks

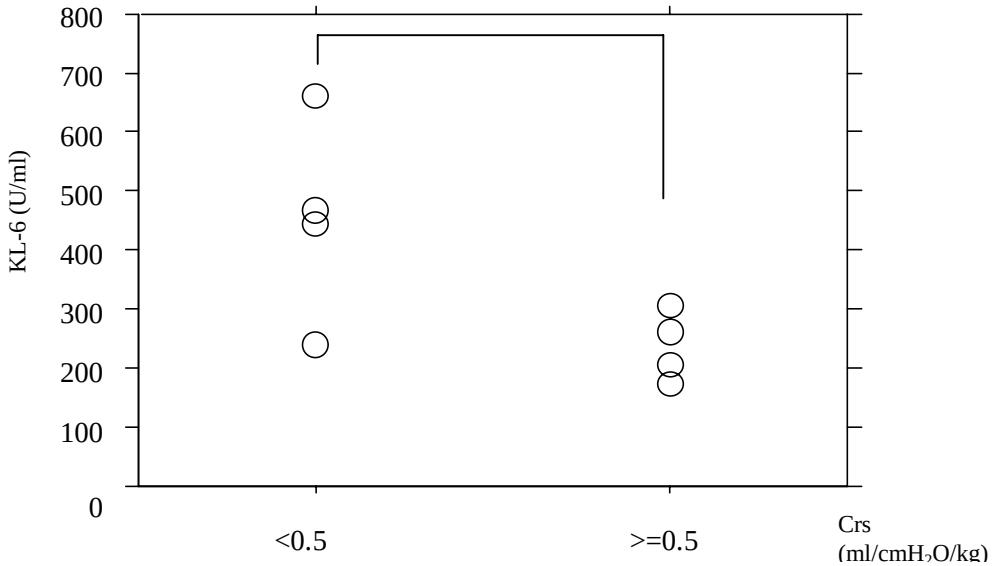

Fig. 4 Peak sKL-6 and Crs in eight ventilated infants for more than 2 weeks old
All infants treated with mechanical ventilation for more than 2 weeks were divided into two groups: lower Crs and higher Crs. Lower Crs was defined as less than 0.5ml/cmH_2O/kg even if occurring only once after 2 weeks of age. Peak sKL-6 values in lower Crs infants were higher than those in high Crs, but there was no significant difference.

could not demonstrate any correlation. In many cases of CLD infants, their respiratory conditions worsen due to various factors (i.e. bronchospasms attack, surfactant deficiency and infection etc.) since 2 weeks of age or later. We also observed similar cases in our CLD group, but sKL-6 levels showed remarkable elevations after 3 or 4 weeks of age in five of 9 CLD cases (Fig .1). The pathogenesis of CLD involved lung injury and abnormal healing (2). The lung injuries mainly occur until 2 to 3 weeks of age, and then the abnormal healing increases gradually. Since KL-6 antigen expresses highly in repaired tissues rather than damaged tissues in the lung (6), sKL-6 levels at 2 to 3 weeks of age were less than 300 U/ml except for one point and those of 4 weeks or later showed wide distribution (Fig. 2).

Three infants in the CLD group received DEX therapy because of deterioration of their respiratory condition, and both their Crs and sKL-6 levels after DEX therapy were higher than before (Fig. 1, Crs data not shown). Takahashi et al. compared sKL-6 levels with adenovirus pneumonia phase in children (13) and found that sKL-6 levels were significantly higher in the recovery than in the acute phase. Takahashi et al. speculated that sKL-6 levels tended to be higher in the recovery phase might be that KL-6 showed high expression levels in regenerated alveolar cell in the remodeling region of interstitial pulmonary disease (13). We also speculated that the elevation of sKL-6 level after the administration of DEX depends on not only the reduction of tissue damage by DEX therapy but also on the enhanced repair of lung tissues. Therefore sKL-6 level may not reflect the clinical course of CLD infant.

For our study, all infants ventilated more than 2 weeks were divided into and evaluated as two groups, one with lower Crs (Crs<0.5ml/cmH_2O/kg) and the other comprising the remaining infants (Fig. 4). Because the infants with Crs of less than 0.5/cmH_2O/kg after 2 weeks of age all underwent some intervention for the treatment of deterioration of the respiratory condition (i.e. DEX or indomethacin) and most infants with respiratory distress have Crs of less than 0.6ml/cmH_2O/kg, our definition of lower Crs seems to be reasonable (12,14). Comparative analysis showed that infants with lower Crs had higher peak sKL-6 levels than did those with higher Crs, but there was no statistically significant difference, although the latter may be the result of the small sample size of our study. The infants with lower Crs usually received some interventions (i.e. high ventilator setting, postnatal steroid, etc.) so that sKL-6 levels of them were likely to be higher than those of infants with higher Crs.

Ogihara et al. compared sKL-6 levels of CLD infants with those of non-CLD infants on day 0-1, 10 and 30 (10). They found that sKL-6 levels of CLD infants were significantly higher than of those without CLD at each time point. Sano et al. have reported similar findings (11). They compared KL-6 levels of serum and alveolar lavage fluid in preterm infants with CLD and without CLD and found that sKL-6 levels of CLD infants were significantly higher than that of non-CLD only on day 14. sKL-6 levels in CLD infants were higher than those in non-CLD on day 14, but there was no statistically significant difference ($p=0.12$, data not shown) in our results. Because one case received DEX later had lower sKL-6 level than 200 U/ml on day 14, sKL-6 levels on day 14 were not always higher than 200 U/ml in CLD infants (Fig.1). We speculated no statistically significant difference was due to small sample size. Both these studies reported that sKL-6 levels of CLD infants were likely to be higher than 200 U/ml after 2 or more weeks of age while those of non-CLD cases were not (10,11). Our results were similar to theirs, except that two of the non-CLD infants had a peak sKL-6 level higher than 200 U/ml while that of one CLD infant was below 200 U/ml. Because one of these non-CLD infants showed evidence of prenatal infection (high IgM value and presence of CAM), it was assumed that inflammation in the pulmonary interstitial tissue might have caused the elevation of sKL-6 level. On the other hand, one CLD infant whose sKL-6 levels were all below 200 U/ml showed no evidence of prenatal infection and could be extubated earlier than other infants in the CLD group. We therefore speculated that this infant's pulmonary tissue was exposed to less lung injuries (i.e. volutrauma, barotrauma and oxidant etc).

KL-6 has been proven to be a useful marker of interstitial lung diseases in adult patients (3, 5, 6, 7, 15). Our results indicate, however, that sKL-6 level is not always a reliable marker of the clinical course of CLD. Arai et al. examined serum surfactant protein D (SP-D) and KL-6 in patients with measles pneumonia (1) and found that serum levels of SP-D decreased immediately after steroid pulse therapy but that sKL-6 levels increased transiently. Serum level of SP-D is also known as a marker of interstitial lung disease, and may be a more accurate marker than sKL-6 level of the clinical course of CLD because of its short half-life (7). On the other hand, our findings suggest that sKL-6 level may be an indicator of the severity of CLD because sKL-6 levels are likely to be higher in more immature infants with a longer duration of mechanical ventilation and reduced pulmonary function (Fig 3). We could find out the significantly negative correlation between GA and sKL-6 levels at both peak and PCA 36 weeks, although the correlation coefficient between GA and sKL-6 level at PCA 36 weeks was low value. The pathogenesis of CLD involved both lung injury and abnormal healing (2). More immature infants are likely to have a more severe form of CLD, require longer ventilation and have more repaired lung tissues so that it

seems reasonable that these more immature infants are likely to have a higher level of sKL-6. We speculate that the elevation of sKL-6 level reflects an amount of repaired lung tissues, but does not deterioration of respiratory condition in CLD infants. Therefore the elevation of sKL-6 level does not always occur when the clinical course of CLD worsened.

In conclusion, we were able to provide evidence of remarkable elevation of sKL-6 in CLD infants, but were unable to detect any relationship between sKL-6 and the corresponding pulmonary function.

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