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# Serum KL-6 Levels in Patients With Pulmonary Complications After Allogeneic Bone Marrow Transplantation

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**Abstract**

KL-6, a mucinous high–molecular weight glycoprotein expressed on type 2 pneumocytes, has been shown to be elevated in the serum and bronchoalveolar lavage fluid of patients with interstitial pneumonitis (IP). We measured the serum levels of KL-6 in patients after they had undergone allogeneic bone marrow transplantation (BMT) to determine whether KL-6 could be a clinically useful indicator for the development of IP. The serum concentrations of KL-6 were determined by a sandwich-type enzyme-linked immunosorbent assay using an anti–KL-6 monoclonal antibody. A total of 1028 samples were tested from 76 patients (78 transplantations) who received BMTs. The KL-6 values were markedly elevated in patients with pulmonary complications, but not in those with acute and chronic graft-versus-host disease, hemorrhagic cystitis, herpes encephalitis, sepsis, and veno-occlusive disease. The serum levels of KL-6 from patients with pulmonary complications were significantly higher than from those without pulmonary complications ($P < .001$) and those with other complications ($P < .001$). Of the 12 patients with pulmonary complications, 6 had idiopathic IP (IIP). The levels were not high at the onset of IIP. Four of 6 IIP patients showed marked elevations of KL-6 levels in parallel with the severity of IP and died of respiratory failure without response to treatment. Assessment of serum KL-6 levels might not be useful for the early diagnosis of IP, but may be a useful indicator for monitoring the severity of IP after BMT. *Int J Hematol.* 2001;74:464-468.


*Key words:* KL-6; Bone marrow transplantation; Interstitial pneumonia

## 1. Introduction

Pulmonary complications are one of the most frequent causes of mortality after bone marrow transplantation (BMT). A total of 20% to 40% of patients develop interstitial pneumonitis (IP) following allogeneic BMT [1-3], and approximately 50% of them die. The symptoms of IP include fever, nonproductive cough, tachypnea, hypoxemia, and diffuse intra-alveolar or interstitial infiltrates on chest radiography. However, those manifestations are not specific and cannot be differentiated readily from viral or fungal pneumonia. Some patients with idiopathic pneumonia present with few symptoms and are diagnosed as having IP incidentally by routine radiography or pulmonary function tests.

KL-6, a mucinous high–molecular weight glycoprotein expressed on type 2 pneumocytes, has been shown to be elevated in the serum and bronchoalveolar lavage fluid of patients with IP [4-6]. In the present study, we measured the serum levels of KL-6 in patients after allogeneic BMT to determine whether KL-6 could be a clinically useful indicator of the disease activity of IP-complicated BMT.

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## 2. Patients and Methods

### *2.1. Patients*

Seventy-six patients with acute leukemia (AL) (n = 32), chronic myelogenous leukemia (CML) (n = 22), myelodysplastic syndrome (MDS) (n = 11), non-Hodgkin's lymphoma

**Table 1.**
Serum Levels of KL-6 in Patients With SAA, AL, CML, and MDS Without Complications After BMT*

| | No. of Samples | Serum KL-6, U/mL (Mean ± SD) |
|---|---|---|
| All | 693 | 214.1 ± 164.2 |
| SAA | 79 | 233.9 ± 415.3 |
| AL | 298 | 196.9 ± 67.4 |
| CML | 213 | 235.2 ± 119.5 |
| MDS | 103 | 204.8 ± 76.1 |

*SAA indicates severe aplastic anemia; AL, acute leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; BMT, bone marrow transplantation.

**Table 2.**
Serum Levels of KL-6 From Patients With Complications After BMT*

| | No. of Cases | Serum KL-6, U/mL (Mean ± SD) |
|---|---|---|
| Acute GVHD | 27 | 202.3 ± 63.3 |
| Hemorrhagic cystitis | 16 | 160.9 ± 59.6 |
| Chronic GVHD | 8 | 213.7 ± 103.2 |
| Herpes encephalitis | 2 | 277.4 |
| Sepsis | 2 | 166.1 |
| VOD | 2 | 143.6 |
| Pulmonary complication | 12 | 753.3 ± 930.3 |

*BMT indicates bone marrow transplantation; GVHD, graft-versus-host disease; VOD, veno-occlusive disease.

(NHL) (n = 1), or severe aplastic anemia (SAA) (n = 10) who received allogeneic BMT were enrolled in this study. The number of transplantations was 78 because 2 patients underwent second transplantations. Their ages ranged from 15 to 50 years (median, 27 years). Forty-one patients were male and 35 were female. All patients in this study gave informed consent.

### *2.2. Measurement of KL-6 Antigen in Serum*

Samples were obtained weekly from pretransplantation to discharge as hospital protocol. The serum concentrations of KL-6 antigen before and after BMT were measured by a sandwich-type enzyme-linked immunosorbent assay using an anti–KL-6 monoclonal antibody (immunoglobulin G1), as previously described [4,7] (Eitest KL-6, Sanko-Junyaku, Tokyo). In brief, polystyrene cups coated with anti–KL-6 antibody were incubated with 0.1 mL of 10-fold diluted serum at 25°C for 1 hour. Then the cups were washed with 0.85% NaCl and incubated at 25°C for 1 hour with 0.1 mL of 1000-fold diluted horseradish peroxidase–conjugated anti–KL-6 antibody. Next, the cups were washed again, and 0.1 mL of ABTS solution (1.5 mg/mL 2,2-azino-bis 3-ethylbenz-thiazoline-6-sulfonic acid), 0.02% $H_2O_2$, and 0.1M citrate buffer at a pH of 4.2 were added, and incubation was performed at 25°C for 1 hour. Finally, 0.013M $NaN_3$ was added to stop the peroxidase reaction, and absorbance at 405 nm was measured. Statistical analyses were carried out with the Student *t* test.

## 3. Results

A total of 1028 samples from 76 patients were tested for serum levels of KL-6. The levels in the samples from patients with SAA, AL, CML, and MDS who did not have any complications such as acute or chronic graft-versus-host disease (GVHD), hemorrhagic cystitis, herpes encephalitis, sepsis, veno-occlusive disease (VOD), or pulmonary complications are shown in Table 1. The mean values of KL-6 levels in each group of patients were less than the cut-off value (500 U/mL) arbitrarily set according to the values from healthy volunteers [8], and there was no significant difference among them. The changes in the average KL-6 levels before and after

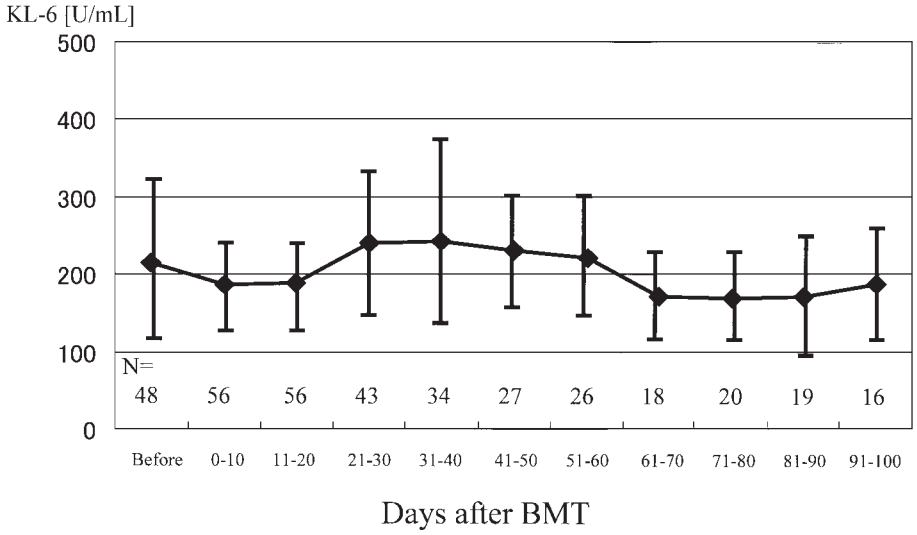


**Figure 1.** Changes in serum KL-6 levels before and after bone marrow transplantation (BMT). Values show median KL-6 levels and SD. N indicates the number of cases in each period.

**Table 3.**
Peak Values of Serum KL-6 in Patients With Pulmonary Complications*

| UPN | Pulmonary Complication | KL-6, U/mL | Outcome |
|---|---|---|---|
| 31 | IIP | 1720 | Died |
| 35 | IIP | 323 | Died |
| 42 | IIP | 1417 | Died |
| 45 | IIP | 262 | Alive |
| 76 | IIP | 2852 | Died |
| 96 | IIP | 3189 | Died |
| 51 | CMV pneumonia | 440 | Died |
| 56 | CMV pneumonia | 427 | Alive |
| 66 | *Pneumocystis carinii* pneumonia | 685 | Died |
| 98 | Obstructive lung disease | 325 | Alive |
| 100 | Obstructive lung disease | 233 | Alive |
| 101 | Radiation pneumonia | 360 | Alive |

*UPN indicates unique patient number; IIP, idiopathic interstitial pneumonitis; CMV, cytomegalovirus.

BMT are shown in Figure 1. The KL-6 values were only elevated in patients with pulmonary complications (Table 2). The values were significantly higher in patients with pulmonary complications than in those without any complications ($P < .001$) and those with complications other than pulmonary ($P < .001$). Table 3 shows the peak value of KL-6 and the outcome for patients with pulmonary complications. Of 12 patients with pulmonary complications, 6 had idiopathic IP (IIP). Of the 6 with IIP, 4 showed markedly high KL-6 levels compared to the patients with other pulmonary complications. All 4 patients with higher levels of KL-6 died of respiratory failure.

Figures 2 and 3 show representative clinical courses of patients with IIP. Figure 2 (unique patient number [UPN] 42) shows the clinical course of an 18-year-old man with acute lymphoblastic leukemia–L2 (by French-American-British [FAB]classification) and Figure 3 (UPN 96) shows that of a 39-year-old woman with acute myelogenous leukemia–M2 (FAB classification). Their initial symptoms were dyspnea and hypoxemia, and their diseases presented as diffuse interstitial shadowing on chest x-ray. The KL-6 values were not unusually high at the onset of IIP, but thereafter they increased gradually. The peak levels in these cases were markedly high, reaching 1417 and 3189 U/mL, respectively, and the patients died without response to high-dose methylprednisolone therapy.

## 4. Discussion

IP represents a group of diseases characterized by diffuse reticulonodular shadowing on chest radiography, typical auscultatory findings, and histological evidence of fibrosing alveolitis [9]. Bronchoalveolar lavage, scintigraphy with $^{67}$Ga, and determination of the serum levels of lactate dehydrogenase may indicate the activity of the disease. However, the findings are not always specific to IP and are often influenced by pulmonary infections [10,11].

KL-6 is a mucin-like glycoprotein expressed on type 2 pneumocytes in alveolar and epithelial cells of bronchioles in the normal human lung [12]. It consists of multiple heterogeneous molecules [13] and is classified as Cluster 9 (MUC 1) of lung tumors and of antigen differentiation [14,15]. KL-6 glycoprotein is expressed strongly on the cell membranes of some malignant cells, such as those of lung adenocarcinoma, pancreatic cancer, and breast cancer. The serum level of KL-6 was elevated in 70% to 100% of patients with IP, including pulmonary fibrosis, hypersensitivity pneumonitis, and radiation pneumonitis [4-6]. Also, KL-6 is a useful marker for the evaluation of IP in patients with collagen diseases [16-18]. Although IP develops frequently

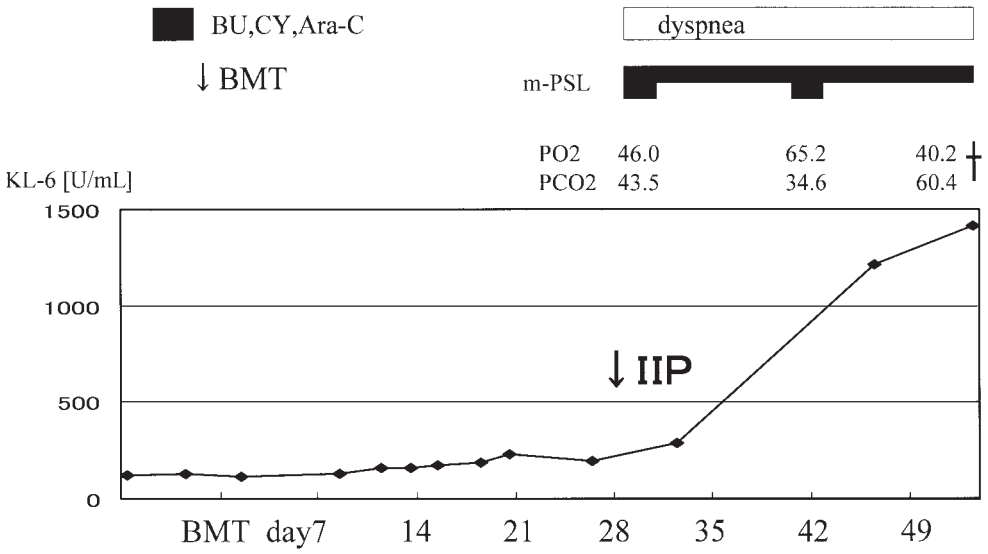


**Figure 2.** Clinical course of patient with unique patient number 42, an 18-year-old man with acute lymphoblastic leukemia–L2 (by French-American-British classification). BU indicates busulphan; CY, cyclophosphamide; Ara-C, cytosine arabinoside; BMT, bone marrow transplantation; m-PSL, methylprednisolone; IIP, idiopathic interstitial pneumonitis. † indicates death.

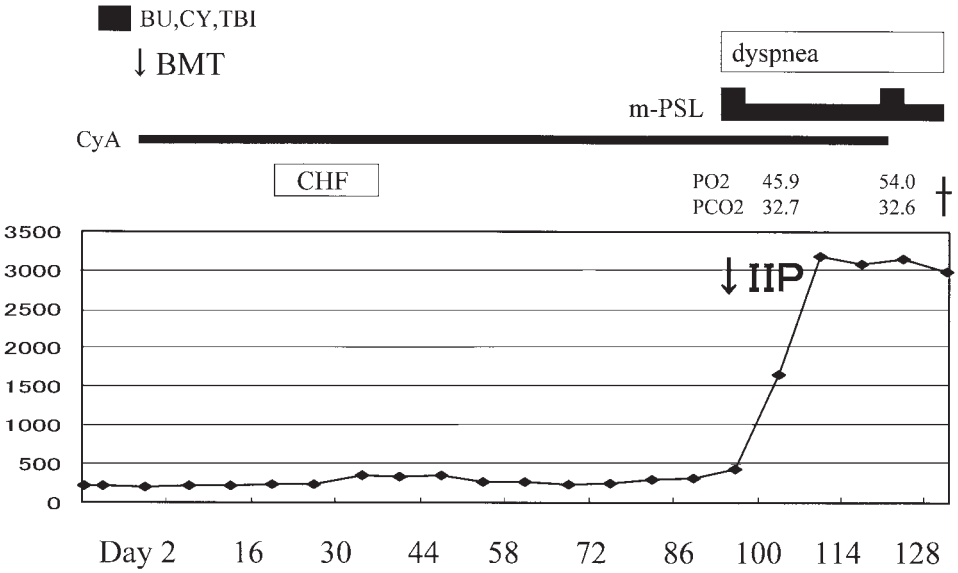


**Figure 3.** Clinical course of patient with unique patient number 96, a 39-year-old woman with acute myelogenous leukemia–M2 (by French-American-British classification). BU indicates busulphan; CY, cyclophosphamide; TBI, total body irradiation; BMT, bone marrow transplantation; m-PSL, methylprednisolone; CyA, cyclosporin A; CHF, congestive heart failure; IIP, idiopathic interstitial pneumonitis. † indicates death.

after BMT, there has been no report on the KL-6 levels of BMT patients. We conducted this study to clarify whether the serum level of KL-6 could be a useful marker for IP in BMT patients.

The serum levels of KL-6 may vary between patients with hematological diseases and those who underwent BMT. There was no significant variation found among the patients with hematological malignant diseases. In addition, serum KL-6 levels in patients without any complications did not change significantly after BMT.

There are various post-BMT complications, including bacterial, fungal, and viral infections, acute and chronic GVHD, VOD, and renal, liver, and pulmonary complications and so on. The KL-6 levels may also be influenced by various complications after BMT; however, no elevation of the levels were observed in patients with acute and chronic GVHD, hemorrhagic cystitis, herpes encephalitis, sepsis, or VOD. The elevations in KL-6 values were specific to pulmonary complications, especially IP. Four of the 6 patients with IIP showed markedly high KL-6 levels. This finding is consistent with previous reports [4-6]. Kobayashi et al [6] reported that the KL-6 levels were significantly higher in patients with pneumonitis (1187 ± 689 U/mL; range, 224 to 2656 U/mL) than in patients without pneumonitis (309 ± 157 U/mL; range, 123 to 855 U/mL). Also, as shown in Figures 2 and 3, KL-6 measurement was not particularly useful for the early diagnosis of IP. However, all patients with high KL-6 values died of IP without response to high-dose methylprednisolone. These outcomes suggest that the KL-6 levels changed in relation to the clinical activity of interstitial lung disease. The study by Kobayashi et al [6] showed that the KL-6 level was significantly higher in patients with clinically active pneumonitis (1497 ± 560 U/mL) compared to that of patients with inactive pneumonitis (441 ± 276 U/mL).

In summary, serum KL-6 levels could be useful indicators for assessing the disease activity of IP following BMT.

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