The Pitfall in Evaluating Several Serological Markers of Pneumocystis carinii Pneumonia in a Neutropenic Patient

Jiro Fujita, Kimihiro Kawakami, Yoshiko Matsuhashi, Shuji Bando, Masami Nagai and Jiro Takahara

Abstract

We compared the significance of several serum markers to evaluate the activity of Pneumocystis carinii pneumonia (PCP) in an immunocompromized patient. We successively measured KL-6, an amino-terminal propeptide of Type III procollagen (PIIINP), and the cytokeratin 19 fragment (CK19) in the sera of a patient with PCP. Interestingly, PIIINP, KL-6, and CK19 levels in the sera did not increase at the time of onset of PCP during a neutropenic phase. Instead, they markedly increased after the recovery of WBC counts. This case suggests that values of PIIINP, KL-6, and CK19 used for monitoring the activity of PCP might be underestimated in neutropenic patients.

(Internal Medicine 39: 659–662, 2000)

Key words: amino-terminal propeptide of Type III procollagen, KL-6, cytokeratin 19 fragment, Pneumocystis carinii pneumonia

Introduction

Pneumocystis carinii pneumonia (PCP) is a life-threatening opportunistic infection associated with suppressed cellular immunity in patients with acquired immunodeficiency syndrome (AIDS) or patients receiving anti-cancer and immunosuppressive therapy (1). Pulmonary infection with Pneumocystis carinii (P. carinii) is associated with histologic findings of interstitial pneumonitis and sometimes irreversible fibrotic changes (2). It has been reported that the severity of HIV-associated PCP is related to the percentage of neutrophilic granulocytes in the bronchoalveolar lavage (BAL) fluid at the time of diagnosis (3, 4). This suggests that inflammatory activity is central in the pathogenesis of PCP (5).

There has been an increasing interest in the identification of serologic markers, which are directly related to the inflammatory process of PCP. One of these is amino-terminal propeptide of Type III procollagen (PIIINP) (5).

KL-6, a human MUC1 mucin, has been reported to be a sensitive serum marker for interstitial pneumonitis (6–8). It has been suggested that KL-6 is released from injured bronchial epithelium and regenerating type 2 pneumocytes (6–8). In addition, Hamada et al reported that the levels of KL-6 in serum are increased in PCP (9).

Cytokeratin 19 fragment (CK19), a well-known marker for lung cancer, also is increased in inflammatory lung diseases including interstitial pneumonia (10). However, there have been no reports which evaluate CK19 in cases of PCP (10).

Considering this background, we attempted to successively measure PIIINP, KL-6, and CK19 levels in the sera of a patient with PCP, and to evaluate the usefulness of these parameters. We unexpectedly found that these serological markers for PCP were not increased during the neutropenic phase, yet were markedly increased after the recovery of white blood cells.

Case Report

A 72-year-old woman who had received treatment of combined chemotherapy for a malignant lymphoma (non-Hodgkin's lymphoma, diffuse, medium, B-cell) for 1 year, was admitted to Kagawa Medical University to receive a combination chemotherapy [vincristine 1 mg/body (day 1, 8, 15), cyclophosphamide 500 mg/body (day 1, 8, 15), methotrexate 25 mg/body (day 1, 8, 15), and oral prednisolone 60 mg/body (day 1–15)] for relapsed malignant lymphoma. Seventeen days after the last treatment of combination chemotherapy, she complained of fever, cough and dyspnea. Fine crackles were heard and a chest radiograph showed bilateral interstitial shadows (Fig. 1). The leukocyte count was 1,300 cells/μl (3.5% lymphocytes). Arterial blood gas analysis at room air showed a hypoxemia with PaO₂ of 53.6 Torr. Anti-cytomegalovirus immunoglobulin M (IgM) antibody was not detectable. She was diagnosed with PCP, clinically. Although methylprednisolone and TMP-SMX were administered followed by the administration of pentamidine isethionate (intravenously and by inhalation), she died due to respiratory failure 29 days after the last treatment of chemotherapy.

To prove the onset of PCP, P. carinii DNA in serum was
Figure 1. Chest X-ray finding at the onset of *Pneumocystis carinii* pneumonia (seventeen days after the last chemotherapy). Bilateral interstitial infiltrates are clearly demonstrated.

successively evaluated by polymerase chain reaction (PCR) for *P. carinii* as described previously (11).

The serum concentration of KL-6 antigen was measured by a sandwich-type enzyme-like immunosorbent assay using KL-6 antibody (12). PIIINP antigenic material was determined by the PIIINP Radioimuno assay Kit (Farmos Diagnostica, Oulu, Finland) (5). CK19 in serum was measured with a two-step sandwich immunoassay using the streptavidin-biotin technique (Enzymun-Test CYFRA21-1; Boehringer Mannheim GmbH, Tutzing, Germany). Clinically, PCP was suggested by chest X-ray finding at day 17 (Fig. 1), and CRP was markedly increased (Fig. 2B). PIIINP, KL-6, and CK19 were successively measured (Fig. 2). In addition, *P. carinii* DNA in sera was demonstrated at day 20, as well as at day 24 (data not shown). However, PIIINP, KL-6, and CK19 were not significantly increased at this point (Fig. 2B, C). In contrast, these parameters became markedly increased after the recovery of white blood cell count (Fig. 2B, C). An autopsy was performed and PCP was confirmed pathologically.

Figure 3 shows the comparison of KL-6 values in patients of PCP with neutropenia (less than 500/µl) and without neutropenia (more than 2,500/µl). It is obvious that KL-6 in sera was not increased in patients with PCP during the neutropenic phase.

Discussion

In our present study, we successively measured PIIINP, KL-6, and CK19 levels in the sera of a patient with PCP and unexpectedly found that these markers did not increase during the neutropenic phase.

It has been reported that serum PIIINP (5) and KL-6 (9, 13) are good markers for evaluating the activity of PCP. PIIINP is removed from Type III procollagen in equimolar fashion by specific proteinases during the formation and growth of Type III collagen fibrils (14). It has been suggested that the PIIINP levels reflect the degree of inflammatory activity of PCP (5). In addition, Hamada et al showed a remarkably elevated level of KL-6, and suggested that KL-6 may be a useful serum marker in the evaluation of PCP in immunocompromised hosts (9).
Markers for *Pneumocystis carinii* Pneumonia

Figure 3. Comparison of KL-6 values in patients of PCP with neutropenia (less than 500/μL) and without neutropenia (more than 2,500/μL). ○ represents patients reported by other investigators and the number of references are listed in the parentheses. ● represents patients treated in our hospital. △ represents the present case during the neutropenic phase as well as after a recovery of neutrophils. All patients were diagnosed with PCP pathologically.

Kawakami et al have also reported that KL-6 might be useful to evaluate the activity of PCP in patients with AIDS (13).

The purpose of our study was to compare the usefulness of three serological markers for PCP. It has been reported that KL-6 is derived from hyperplastic type II pneumocytes in PCP (9). In addition, it has been reported that CK19 is also derived from hyperplastic type II cells in patients with interstitial pneumonia (15). In contrast, it has been reported that PIIINP is derived from mesenchymal cells, especially from granulation tissues (5). Elevation of these markers in PCP suggests that these cells play a role in the disease process of PCP.

However, we unexpectedly found that these serological markers did not increase during the neutropenic phase. This observation suggests that elevation of PIIINP, KL-6, and CK19 required proteases released from neutrophils. It has been reported that inflammatory activity especially the activation of neutrophils may be central in the pathogenesis of HIV-related PCP (5). The amount of *P. carinii* on the alveolar lumen is correlated with the amount of neutrophilic granulocytes in the interstitium (16), and furthermore, increased percentages of neutrophils in BAL fluid are inversely correlated with arterial oxygenation (16, 17) and are associated with an increased risk of a fatal outcome (3, 4).

In addition, Nakamura et al demonstrated that BALF from patients with chronic airway inflammatory disease contains higher levels of neutrophils and neutrophil elastase activity than BALF from nonsmoking controls, and that the number of neutrophils in BALF are significantly correlated with the levels of CK19 in BALF (10). They also demonstrated that CK19 is released from bronchial epithelium by neutrophil-derived injurious substances (10).

There are few reports which have evaluated the relationship between proteases derived from neutrophils and the elevation of KL-6 in serum. However, Kohno et al reported that KL-6 levels in BALF are significantly correlated with numbers of neutrophils and lymphocytes (6). Since injured type 2 pneumocytes are the major supplier of soluble KL-6, pulmonary vascular leakage caused by neutrophil influx might be the main factor of elevation of KL-6 in serum in interstitial lung diseases and PCP.

There are few reports which have evaluated PIIINP and neutrophils. Harrison et al have reported increased PIIINP in BALF of lung disease in systemic sclerosis (18). They also suggested that pulmonary vascular leakage and neutrophil influx may be early pathological features of lung disease in systemic sclerosis and these are frequently associated with enhanced collagen production (18). Gilligan et al have reported increased neutrophils, collagenase, and PIIINP in the bronchoalveolar lavage fluid of patients with severe rheumatoid lung disease (19). They also suggested that collagenase derived from neutrophils plays a role in the synthesis of PIIINP (19).

These evidences and the present case suggest that elevation of PIIINP, KL-6, and CK19 require proteases released from neutrophils. This hypothesis should be evaluated in future studies. In addition, since these three markers had changed in the same manner, it is necessary to use only one of these three markers for monitoring the activity of PCP.

Although the possibility that prednisolone included in the chemotherapeutic regimen affected the clinical course of this case could not be excluded, this was unlikely because there was no evidence of PCP before and during combination chemotherapy.

In summary, the present case suggests that KL-6, PIIINP, and CK19 levels in the sera of a patient with PCP did not increase at the time of onset of PCP during a neutropenic phase. Therefore, clinicians should consider that values of PIIINP, CK19, and KL-6 used for monitoring the activity of PCP might be underestimated in neutropenic patients.

**References**


