CYFRA 21-1, a cytokeratin subunit 19 fragment, in bronchoalveolar lavage fluid from patients with interstitial lung disease

Hiroshi KANAZAWA, Takahiro YOSHIKAWA, Masashi YAMADA, Seiichi SHOJI, Tatsuo FUJII, Shinzoh KUDOH, Kazuto HIRATA and Junichi YOSHIKAWA
The First Department of Internal Medicine, Osaka City University Medical School, 1-5-7, Asahi-machi, Abenoku, Osaka 545, Japan

1. It has been suggested that CYFRA21-1, a cytokeratin subunit 19 fragment, is potentially useful for diagnosis and monitoring of lung carcinoma. However, serum levels of CYFRA21-1 are also increased in a high proportion of patients with interstitial lung disease. In this study we measured CYFRA21-1 levels in bronchoalveolar lavage fluid from 10 normal subjects, 18 patients with idiopathic pulmonary fibrosis and 14 patients with sarcoidosis, and determined whether any relationship exists between CYFRA21-1 levels in bronchoalveolar lavage fluid and clinical parameters.

2. CYFRA21-1 levels in bronchoalveolar lavage fluid were significantly higher in patients with sarcoidosis (mean value 8.3 ng/ml, \(P < 0.01\)) and idiopathic pulmonary fibrosis (42.5 ng/ml, \(P < 0.005\)) than in normal controls (1.0 ng/ml). Moreover, higher CYFRA21-1 levels in bronchoalveolar lavage fluid were found in sarcoidosis patients in radiological stage 2 or 3 than in those in stage 1. In patients with idiopathic pulmonary fibrosis, there was a significant correlation between CYFRA21-1 levels, and percentage of inflammatory cells in bronchoalveolar lavage fluid (\(r = 0.56, P < 0.05\)) and the magnitude of the alveolar—arterial oxygen pressure difference \(P(A-a)O_2\) gradient (\(r = 0.66, P < 0.01\)).

3. Serial bronchoalveolar lavage samples were obtained from six patients with clinically active pneumonitis after they had undergone systemic corticosteroid therapy. CYFRA21-1 levels were significantly lower after these patients exhibited clinical improvement (\(P < 0.05\)).

4. These findings suggest that the level of CYFRA21-1 in bronchoalveolar lavage fluid is a useful marker for the clinical diagnosis of pneumonitis, and is also adequate for the evaluation of disease activity, especially over the course of treatment.

INTRODUCTION

Cytokeratins are intermediate filaments which form part of the cytoskeleton of various epithelial tissues [1]. Immunohistochemical studies using broad-spectrum cytokeratin antibodies have demonstrated the expression of cytokeratins in both normal lung and lung tumours [2]. These cytokeratins are not randomly distributed in the various epithelia, but rather appear to be characteristic of certain types of epithelial differentiation. The presence of a specific subtype of cytokeratin, corresponding to a particular type of differentiation, is detectable at the cellular level with monoclonal antibodies [3]. The family of human cytokeratins consists of 19 different polypeptides, which have been numbered 1 to 19. Cytokeratin subunit 19 is expressed on type 1 and 2 pneumocytes and respiratory bronchiolar epithelial cells in normal lung. A fragment of cytokeratin subunit 19 can be measured using two mouse monoclonal antibodies, KS19-1 and BM19-21, and this fragment is referred to as CYFRA21-1. It has recently been suggested that CYFRA21-1 is potentially useful for diagnosis and monitoring of lung carcinoma, especially for squamous cell carcinoma [4].

Serum levels of CYFRA21-1 are increased in a high proportion of patients with interstitial lung disease [5]. We speculated that the increased CYFRA21-1 in serum from patients with interstitial lung disease is derived from damaged or regenerating epithelial cells in the lower respiratory tract. However, since CYFRA21-1 is also expressed on various epithelial cells, it is not clear whether the increased CYFRA21-1 is certainly derived from the epithelial cells in the lower respiratory tract. In this study, we measured CYFRA21-1 levels in bronchoalveolar lavage fluid (BALF) from normal subjects and patients with interstitial lung disease, including idiopathic pulmonary fibrosis (IPF) and sarcoidosis, and determined the relationship which exists between CYFRA21-1 levels in BALF and clinical parameters.

METHODS

Study subjects

This study was prospective and consecutive, and healthy controls were volunteers from our hospital.
A total of 18 patients with IPF (64 ± 8 years, mean ± S.D.; 14 male and 4 female), 14 patients with sarcoidosis (45 ± 5 years, 8 male and 6 female), and 10 healthy control subjects (51 ± 8 years, 8 male and 2 female) were enrolled in the study. IPF was diagnosed by clinical examination, standard chest roentgenography, computed tomographic scan of the chest, pulmonary function tests and examination of lung tissue obtained on transbronchial biopsy. The diagnosis of sarcoidosis was supported by the presence of epithelioid cell granulomas in organs on which biopsies were performed, including skin, lymph nodes and lung, and by clinical features. As classified by radiological stage of sarcoidosis [6], four patients were in stage 1 (bilateral hilar adenopathy with or without mediastinal and paratracheal lymph node enlargement), four patients in stage 2 (hilar adenopathy with pulmonary infiltrates), and three patients in stage 3 (pulmonary infiltrates without lymphadenopathy). Eight of the healthy control subjects, 12 patients with IPF and five patients with sarcoidosis had a history of smoking. Pulmonary function test was performed in all patients.

**Bronchoalveolar lavage (BAL)**

After obtaining informed consent for participation, BAL was performed using flexible fibre-optic bronchoscopes (Olympus, Tokyo, Japan) gently wedged in either a singular or right middle lobe subsegmental bronchus. No patients were endotracheally intubated at the time of the procedure. Sterile 0.9% NaCl was used as the instillate. A total of 150 ml of fluid was instilled. The fluid was infused in three 50 ml aliquots from a syringe. After each aliquot was infused, it was immediately recovered by hand suction into the syringe. The collected lavage material was pooled and filtered through a sheet of sterile surgical gauze and then centrifuged at 400 g for 10 min to separate the cellular and non-cellular components of the BALF. The number of cells was determined by washing the recovered cell pellet with Krebs-buffer solution and counting with a haemocytometer. The absolute number of cells was expressed as cells \( \times 10^9 \) per millilitre of unconcentrated lavage fluid. Differential cell counts were determined from cell suspensions displayed on slides made by Cytospin. The cells on a slide were air-dried and stained by Wright–Giemsa solution. The differential counts were performed by examining at least 200 cells using a standard light microscope. Albumin in BALF was measured by a turbidimetric immunoassay using microalbumin kits (Nitto Boseki Co., Ltd., Tokyo, Japan).

**CYFRA21-1 assay**

The supernatant of BALF was removed without disturbing the cell pellet and kept frozen at \(-80^\circ C\). CYFRA21-1 was determined with a two-step sandwich ELISA kit (Enzyme-Test CYFRA21-1, Boehringer–Mannheim, Germany). With this method, cytokeratin 19 is recognized by two mouse monoclonal antibodies, directed against two different epitopes of a fragment of cytokeratin subunit 19. This ELISA kit consistently detects CYFRA21-1 at a concentration above 1 ng/ml.

**Statistical analysis**

Values are expressed as the mean ± S.D. Multiple comparisons of the data were performed with the Kruskal–Wallis non-parametric one-way analysis of variance. When significant, the differences between groups were further compared with the non-parametric Mann–Whitney U-test. Wilcoxon’s signed rank test was used to compare values before and after corticosteroid therapy. Spearman’s rank-correlation coefficients were determined for analysis of correlation between variables. Statistical significance was defined as \( P < 0.05 \).

**RESULTS**

Characteristics of BAL analysis for each group are shown in Table 1. Recovery rates of BAL were significantly lower for patients with IPF (51 ± 8.5%) than for healthy normal controls (62 ± 7.5%). In patients with sarcoidosis, total cell count and percentage of lymphocytes were significantly higher than in normal controls. In patients with IPF, percentages of lymphocytes and polymorphonuclear leucocytes were significantly higher than in normal controls.

CYFRA21-1 levels in BALF were significantly higher in patients with sarcoidosis [mean 8.3 (0–27) ng/ml, \( P < 0.01 \)] and IPF [42.5 (0–180) ng/ml, \( P < 0.005 \)] than in normal controls [1.0 (0–3.3) ng/ml] (Figure 1). Moreover, higher levels of CYFRA21-1 in BALF were found in seven patients with radiological stage 2 or 3 (mean 12.7 ng/ml, \( P < 0.01 \)) than in seven patients with radiologic stage 1 (3.8 ng/ml). However, smoking history had no significant effect on CYFRA21-1 levels. In patients with IPF, there was a significant correlation between CYFRA21-1 levels and percentage of inflammatory cells in BALF (\( r = 0.56, P < 0.05 \)) (Figure 2). We recorded the alveolar–arterial oxygen pressure difference (\( P(A-a)O_2 \)) in room air at the time of BAL. A significant correlation was found between CYFRA21-1 levels in BALF and the magnitude of the \( P(A-a)O_2 \) gradient (\( r = 0.66, P < 0.01 \)) (Figure 3). However, we did not find any correlations between CYFRA21-1 levels and pulmonary function data. Serial BAL samples were obtained from six patients with clinically active pneumonitis (four with IPF, two with sarcoidosis) after they had undergone systemic corticosteroid therapy. Figure 4 shows the changes in CYFRA21-1 levels in these patients. CYFRA21-1 levels were significantly lower...
Table I  Characteristics of BAL analysis

<table>
<thead>
<tr>
<th></th>
<th>Recovery rate (%)</th>
<th>Total cell count (x 10^4/ml)</th>
<th>Pulmonary alveolar macrophage</th>
<th>Lymphocyte</th>
<th>Polymorphonuclear leucocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls (n = 10)</td>
<td>62 (7.5)</td>
<td>1.52 (0.48)</td>
<td>92 (5.8)</td>
<td>6.8 (5.5)</td>
<td>1.2 (2.0)</td>
</tr>
<tr>
<td>Sarcoidosis (n = 14)</td>
<td>60 (5.8)</td>
<td>2.75 (0.82)^*</td>
<td>81 (8.7)</td>
<td>17 (9)**</td>
<td>1.8 (0.9)</td>
</tr>
<tr>
<td>IPF (n = 18)</td>
<td>51 (8.5)^*</td>
<td>2.18 (1.04)</td>
<td>84 (8.0)</td>
<td>9.4 (5.7)^*</td>
<td>6.3 (3.2)**</td>
</tr>
</tbody>
</table>

Figure 1  CYFRA21-1 levels in BALF from normal controls, patients with sarcoidosis and patients with IPF

Discussion

CYFRA21-1 levels were increased significantly in BALF from patients with pneumonitis, as previously reported for serum [5]. CYFRA21-1, a fragment of
cytokeratin, is expressed in bronchiolar epithelial cells and pneumocytes, and might be released in association with lysis or regeneration of these cells. In pneumonitis, activated inflammatory cells induce the degradation of cytokeratin, resulting in the release of large amounts of cytokeratin fragments in the lower respiratory tract, consistent with the findings of the present study of CYFRA21-1 as a marker of interstitial pneumonitis. However, to our knowledge, sensitive markers of disease activity in pneumonitis have not been established. Chest radiography, lung function testing, blood gas analysis and determination of serum C-reactive protein and lactate dehydrogenase levels are usually performed during management of this disease. The information provided by these markers is generally non-specific, and is often influenced by infection or inflammatory processes affecting other organs. 67Ga scintigraphy is a useful technique for assessing the activity of inflammatory processes such as pneumonitis, but Keogh et al. [7] demonstrated that findings with this technique often remain positive after the disease has become clinically inactive. Accordingly, it appears that the confusing results of 67Ga scintigraphy may be ascribed to the lack of an accurate method of measurement of disease activity in pneumonitis. Increased numbers of inflammatory cells in BALF reflect the activity in patients with pneumonitis, such as those with IPF [8] and sarcoidosis [9].

In this study, inflammatory cells in BALF were significantly increased in patients with sarcoidosis and IPF. Based on the findings of cellular infiltration, patients with sarcoidosis and IPF had active pneumonitis to various degrees. A total of 14 patients with histologically confirmed sarcoidosis were studied to ascertain the usefulness of CYFRA21-1 levels as a marker of active pneumonitis in sarcoidosis. Those patients with sarcoidosis classified as radiographic type 2 or 3 had significantly higher levels of CYFRA21-1 in BALF than those with type 1. We also found that in patients with IPF, CYFRA21-1 levels in BALF were correlated with percentages of inflammatory cells and the magnitude of the P(A-a)O2 gradient. In addition, CYFRA21-1 levels decreased with clinical improvement of pneumonitis after systemic corticosteroid therapy. Two patients with the highest levels of CYFRA21-1 in BALF died of progressive respiratory failure. These findings suggest that CYFRA21-1 levels in BALF may be a marker of pneumonitis. Levels of CYFRA21-1 change with disease activity and after treatment. It is possible that changes in CYFRA21-1 levels in BALF could be used to monitor disease activity by serial lavage in patients. However, in practice, few patients undergo serial lavage and, given the small number of patients in the current study undergoing serial lavage, it is unclear whether useful prognostic information could be obtained by measuring serial CYFRA21-1 levels in BALF. It is important to determine whether CYFRA21-1 is a more useful marker of pneumonitis than other recently identified markers such as type III procollagen N-terminal peptide [10], KL-6 [11] and progastrin-releasing peptide [12].

In conclusion, CYFRA21-1 levels in BALF were measured in patients with IPF and sarcoidosis, and it was shown that CYFRA21-1 was useful for the diagnosis of pneumonitis, evaluation of disease activity, and assessment of response to treatment. However, a much more extensive study involving longer follow-up and greater numbers would be required to define the sensitivity and specificity of CYFRA21-1 levels in BALF for diagnostic or prognostic purposes in interstitial lung disease. These findings suggest that CYFRA21-1 levels in BALF may be a useful marker in pneumonitis.

REFERENCES


Received 10 June 1997/5 January 1998; accepted 6 January 1998