**Title page**

**Title:**

# Lung Miliary Micro-nodules in Human T-cell Leukemia Virus Type I Carriers

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**Running Title:**

Lung Micro-nodules in HTLV-1 carriers.

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**Abstract:**

Human T-cell leukemia virus type 1 (HTLV-1) carriers rarely accompany inflammatory disorders in multiple organs besides the well-known complication, adult T-cell leukemia/lymphoma (ATLL). HTLV-1 associated bronchiolo-alveolar disorder (HABA) has been proposed as an immune mediated pulmonary reaction rarely seen in HTLV-1 carriers. The reported clinico-pathological patterns of HABA were diffuse panbronchiolitis (DPB) and lymphoid interstitial pneumonia (LIP).

We report three cases of HTLV-1 carriers showing miliary micro-nodules throughout both lungs. Microscopic examination in the VATS biopsies demonstrated that all have multiple discrete micro-nodules which consisted of marked lymphoid infiltration, granulomas, eosinophils and a few foci of necrosis inside the granuloma. No findings indicating ATLL, other neoplastic conditions, infection, or interstitial pneumonia including DPB and LIP were found after panels of special staining and immunohistochemical examinations. Two patients improved without treatment within one month, with no evidence of recurrence after 7 years. One patient showed slow deterioration of lung reticular shadows in spite of a low dose corticosteroid therapy (Prednisolone 10mg/day). We believe these cases may be a new recognized variant of HABA.

**Key words:**

Human T-cell leukemia virus type-1, HABA, Lymphoproliferative Disorders, case reports,

**Abbreviation list**

ATLL= adult T-cell leukemia/lymphoma

ATLA = adult T-cell leukemia-associated antigen

CT = computed tomography

DPB = diffuse panbronchiolitis

EBV = Epstein-Barr virus

EBER = Epstein-Barr encoded RNA associated protein

HP = hypersensitivity pneumonitis

HTLV-1 = human T-cell leukemia virus type 1

HABA = HTLV-1 associated bronchiolo-alveolar disorder

LDH = lactate dehydrogenase

LIP = lymphoid interstitial pneumonia

LYG = Lymphomatoid granulomatosis

PCR = polymerase chain reaction

VATS = video assisted thoracic surgery

## Introduction:

HTLV-1 is a retrovirus which infects 10 to 20 million people worldwide.[1] Despite this prevalence, especially in some endemic areas such as southwestern Japan, Caribbean, and South America, HTLV-1 is associated with disease in approximately 5% of infected individuals.[2] HTLV-1 not only causes ATLL but also other inflammatory disorders, including myelopathy, tropical spastic paraparesis and bronchopneumopathy.[3,4] "HTLV-1 Associated Bronchiolo-Alveolar Disorder (HABA)" was proposed as an immune mediated pulmonary reaction by Kimura et al in 1989. Similar conditions were also reported as HTLV-1 associated bronchopneumopathy (HAB)[5,6] and HTLV-1 associated pneumopathy[7]. Most cases look identical, and the differences of conditions among those conditions are unclear. In the current report, we selected to use the term HABA. Several additional papers indicate "HABA" has two major histological patterns, one is DPB (diffuse panbronchiolitis) and the other is LIP (lymphoid interstitial pneumonia). However the disease entity is not completely established.[8,9] Here, we describe unique three cases seen in HTLV-1 carriers.

## Cases Reports

### Case 1

A 75 year-old male ex-smoker complained of cough and sputum production during his follow up of his myocardial infarction in our hospital. The cough and sputum did not improve in the 5 months before admission regardless of taking the expectorants. A thoracic CT scan showed bilateral miliary micro-nodules, pleural nodules and interlobular septal thickening (Fig1). The serum test revealed that he was positive for antibodies against HTLV1 and had highly elevated sIL-2R, slightly elevated LDH and KL-6. His peripheral blood had no atypical lymphocytes indicating the presence of ATLL. Bronchoalveolar lavage from right B4b indicated increase of the lymphocytes (CD4/CD8=3.51) and atypical cells were not seen(Table1). One month later, video assisted thoracic surgery (VATS) was performed. After confirming the final diagnosis, the patient was treated with low dose prednisone, starting with 10mg/body per day. Even though low-dose corticosteroid therapy was continued for 5 years, chest reticular shadows gradually worsened.

## Case2

A 65 year-old male ex-smoker complained of a continuous dry cough, particularly worse in the mornings for 5 years. Six months following his first admission, he produced yellow sputum. Malignant neoplasm was not detected and serum tests for collagen vascular disease were positive for RNP, SS-A, SS-B, Scl-70 and Jo-1. The serum test was positive for anti-body against HTLV-1. Bronchoalveolar lavage from right B8b demonstrated elevated lymphocytes (CD4/CD8=4.53) (Table1). VATS biopsy from right S3b and S6 was performed. Symptoms and nodular shadow spontaneously improved without treatment. The patient has been asymptomatic for 9 years.

## Case3

A 70 year old male ex-smoker was admitted with a dry cough. A Chest CT revealed miliary lesions and the serum test was positive for HTLV1 antibody. The serum level for sIL-2R was highly elevated along with a slight elevation of LDH and KL-6, but peripheral blood showed no atypical lymphocytes. Bronchoalveolar lavage from right B5 demonstrated elevated lymphocytes (CD4/CD8=3.03) (Table1). The VATS biopsy was performed to establish the diagnosis. The patient spontaneously recovered without

treatment within 1 month. Three years following his first admission, the patient experienced recurrence of symptoms. Although the patient again spontaneously recovered without treatment within 1 month, five years after his first admission, the patient experienced another recurrence. The patients symptoms resolved following treatment with low dose erythromycin and inhaled corticosteroids. The patient has been asymptomatic for the past 7 years.

Serum testing in all cases were positive for HTLV1 antibody, no atypical lymphocytes or ATLL in the peripheral blood were identified in any cases. Eosinophilia was not seen in all cases. Proviral DNA for HTLV1 was not detected in any biopsied specimens.

## Radiological Findings:

The radiological findings of these cases are summarized at Table2. All cases showed miliary micro-nodules, up to 3mm in size and located randomly in both lungs. One case had emphysema and two cases had bronchiectasis. Neither ground glass opacity nor consolidation was seen in any of the cases.

**Pathological Findings:**

The histological findings of the three cases are summarized in Table3. The micro-nodules less than 3mm in diameter were located mainly around the airways, but sometimes in the periphery of the secondary lobule. (Figure 2a, case1) These nodules consist of severe lymphoid infiltration, florid tissue eosinophilia and epithelioid granulomas. (Figure2b-d) These small epithelioid granulomas were vaguely demarcated and mostly located in the interstitium. Furthermore these granulomas were made up of thin epithelioid hystiocytes and contained scant giant cells. Although most granulomas were non-necrotizing, there were a few small foci of necrosis inside the granuloma in Case 1 and 2. (Figure 2c) No cases showed cellular atypia. Around the micro-nodules, there were polypoid organizations inside the airspace. No acid-fast organism was found when using Ziehl-Neelsen stains. The Groccot's methenamine silver stains and the gram stains were all negative as well. None of the cases demonstrated evidence of EB virus infection, as determined by EBER in situ hybridization. The immunohistochemistory showed CD3 lymphocytes were more predominantly infiltrated than the CD20 lymphocytes. CD4 positive cell were major

components of these infiltrations.

## Discussion:

HTLV-1 Associated Bronchiolo-Alveolar Disorder (HABA) is a proposed immune mediated pulmonary reaction by Kimura et al in 1989. Some previous reports described that HABA has two major morphological patterns of DPB and LIP.[8,10] Interestingly, the report of HABA is mostly limited within Japan, although other reactions including HAM is known to affect HTLV-1 carriers worldwide.

Several types of CT findings in HTLV-1 carriers were reported, such as LIP pattern,[8,10] centrilobular nodules[8] and randomly distributed micronodules.[9] Okada et al. described centrilobular nodules being a common pattern of CT findings in HTLV-1 carriers.[11] In his study, chest CT scans revealed abnormalities in 40.7% with HTLV-1, with centrilobular nodules especially tree-in-bud nodularity the most frequent findings. He also proposed a correlation between CT scans and pathological findings.[10] These centrilobular nodules were represented by lymphocytic infiltration distributed along respiratory bronchioles. Our three cases showed miliary

micronodules randomly located in both lungs in the CT. Histological feature of micronodules which consisted of severe lymphoid infiltration was similar, but they were located not only along the airway, but also in periphery of the secondary lobule.

Ishii reported a case with miliary micro-nodules similar to ours with micronodular lesions containing necrotizing and non-necrotizing epithelioid cell granulomas with fibrin and intraluminar organization and various types of inflammatory cell infiltration, including eosinophils.[9] In our cases, no microorganisms, including parasites, were observed in these granulomatous lesions, using HE, Grocott's methenamine silver and Ziel-Nielsen stains. PCR for detecting mycobacteria were negative for all cases. Although the symptoms were mild, pathologic features were relatively marked especially in showing a few foci of necrosis and airspace organization. Prognosis seems excellent, but some currently unknown factor may lead slow progression as case 1 experience.

Differential diagnosis of current series includes infection, lymphoma, hypersensitivity pneumonitis, and sarcoidosis. Fujii et al. reported a case report similar to our series in which they emphasized that the features of

granuloma seen in the reported case are different from sarcoidosis[12]. As they indicated in their report, granulomas seen in our series were loose and associated with numerous lymphocytosis showing damage of vascular wall. Presence of rare necrotizing granuloma is also different from sarcoidosis granuloma.

Utsunomiya et al. reported that peripheral eosinophilia was a poor prognostic marker of the ATLL patient.[11] He also reported that eosinophilia was significantly associated with serum IL-5. Ishii guessed that eosinophilia may have played some role in the bronchiolo-alveolar disorder, and may have some kind of infection or allergic reaction, such as hypersensitivity pneumonitis(HP).[9] His patient improved without treatment, this fact may prove his hypothesis. In our cases, we did not detect high serum IL-5 levels nor eosinophilia in the peripheral blood, but the tissue eosinophilia. The clinical course with spontaneous remission raises the possibility of an allergic reaction like HTLV-1-associated bronchioloalveolopathy.

Kadota et al, in his study of comparing HABA with DPB, suggested that the radiological features of these two conditions were quite similar.[13] DPB principally affected the respiratory bronchiole causing a severe obstructive

respiratory disorder. Histologically, inflammation of DPB was characterized by prominent interstitial vacuolated or foamy macrophages infiltrate around the bronchiole.[14] In our cases, nodules were randomly located which is not only around the bronchioles but also at the periphery site of the secondary lobule. Tissue eosinophilia and granulomas with rare central necrosis were not the features of DPB as well.

Further accumulation of clinical, radiological and pathological information is necessary to determine how to treat with patients whose symptoms are mild as seen in our 3 cases.

As mentioned above, histology of current HABA cases are not consistent with DPB or LIP. Our cases may be another variant of HABA, such as Ishii suggested HTLV-1-associated bronchioloalveolopathy. The proliferation of T-cells, tissue eosinophilia and presence of granulomas are suspicious of an immunological reaction. Along with HTLV-1 infection, like HIV carrier,[12] immunological disorders may occur in the lung.

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References


1 de The G, Bomford R. An HTLV-I vaccine: why, how, for whom? AIDS Res Hum Retroviruses 1993; 9:381-386

2 Cleghorn FR, Manns A, Falk R, et al. Effect of human T-lymphotropic virus type I infection on non-Hodgkin's lymphoma incidence. J Natl Cancer Inst 1995; 87:1009-1014

3 Steinfort DP, Brady S, Weisinger HS, et al. Bronchiectasis in Central Australia: A young face to an old disease. Respir Med 2007

4 Yoshioka R, Yamaguchi K, Yoshinaga T, et al. Pulmonary complications in patients with adult T-cell leukemia. Cancer 1985; 55:2491-2494

5 Uji M, Matsushita H, Watanabe T, et al. [A human T-cell lymphotropic virus type 1 carrier presenting with Sjogren's syndrome and bronchopneumopathy]. Nihon Kokyuki Gakkai Zasshi 2006; 44:1011-1015

6 Maruyama I, Mori S, Kawabata M, et al. [Bronchopneumonopathy in HTLV-1 associated myelopathy (HAM) and non-HAM HTLV-1 carriers]. Nihon Kyobu Shikkan Gakkai Zasshi 1992; 30:775-779

7 Watanabe T. HTLV-1-associated diseases. Int J Hematol 1997; 66:257-278

8 Sugisaki K, Tsuda T, Kumamoto T, et al. Clinicopathologic characteristics of the lungs of patients with human T cell lymphotropic virus type 1-associated myelopathy. Am J Trop Med Hyg 1998; 58:721-725

9 Setoguchi Y, Takahashi S, Nukiwa T, et al. Detection of human T-cell lymphotropic virus type I-related antibodies in patients with lymphocytic interstitial pneumonia. Am Rev Respir Dis 1991; 144:1361-1365

10 Ishikawa N, Awaya Y, Maeda H, et al. KL-6 as an indicator for lymphocytic interstitial pneumonia (LIP) in a human T-lymphotrophic virus type 1 (HTLV-1) carrier. Ann Hematol 2002; 81:474-477

11 Okada F, Ando Y, Yoshitake S, et al. Pulmonary CT findings in 320 carriers of human T-lymphotropic virus type 1. Radiology 2006; 240:559-564

12 Fujii M, Sumi Y, Atarashi K, et al. Muscle weakness in extremities and diffuse centrilobular nodules in lungs. HTLV-1-associated bronchiolo-alveolar disorder. Thorax 2011; 66:546-547, 552

13 Kadota J, Mukae H, Fujii T, et al. Clinical similarities and differences between human T-cell lymphotropic virus type 1-associated bronchiolitis and diffuse panbronchiolitis. Chest 2004; 125:1239-1247

14 Iwata M, Colby TV, Kitaichi M. Diffuse panbronchiolitis: diagnosis and distinction

from various pulmonary diseases with centrilobular interstitial foam cell accumulations. Hum Pathol 1994; 25:357-363

**Figures:**

Figure 1. Chest CT scan from case 1 shows micro-nodular lesion distributed at random.

Figure 2. Histopathological features of Case 1. a) The micro-nodules are miliary distributed in the secondary lobules. Lymphocyte infiltration is patchy. b) Small granulomas (arrows) mostly without necrosis are identified inside the micro-nodules. c) Rare foci of tiny necrosis (arrow head) are identified inside the granuloma. Note palisading histiocytes (arrows) around necrosis. Non-necrotizing granuloma (asterisk) is also found inside the same nodule. d) Marked tissue eosinophilia is found in some nodules.

Figure 1.

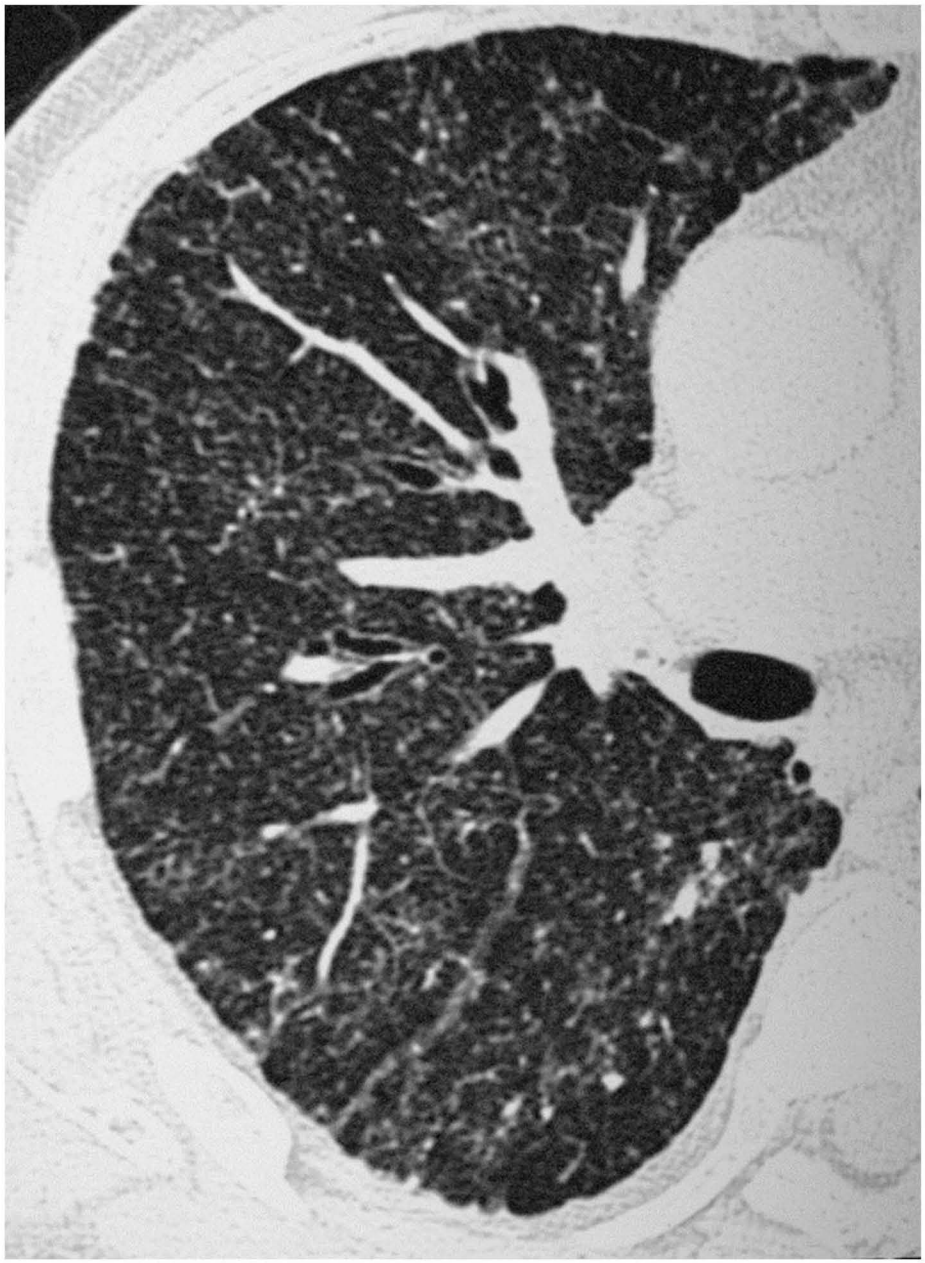

Figure 2.

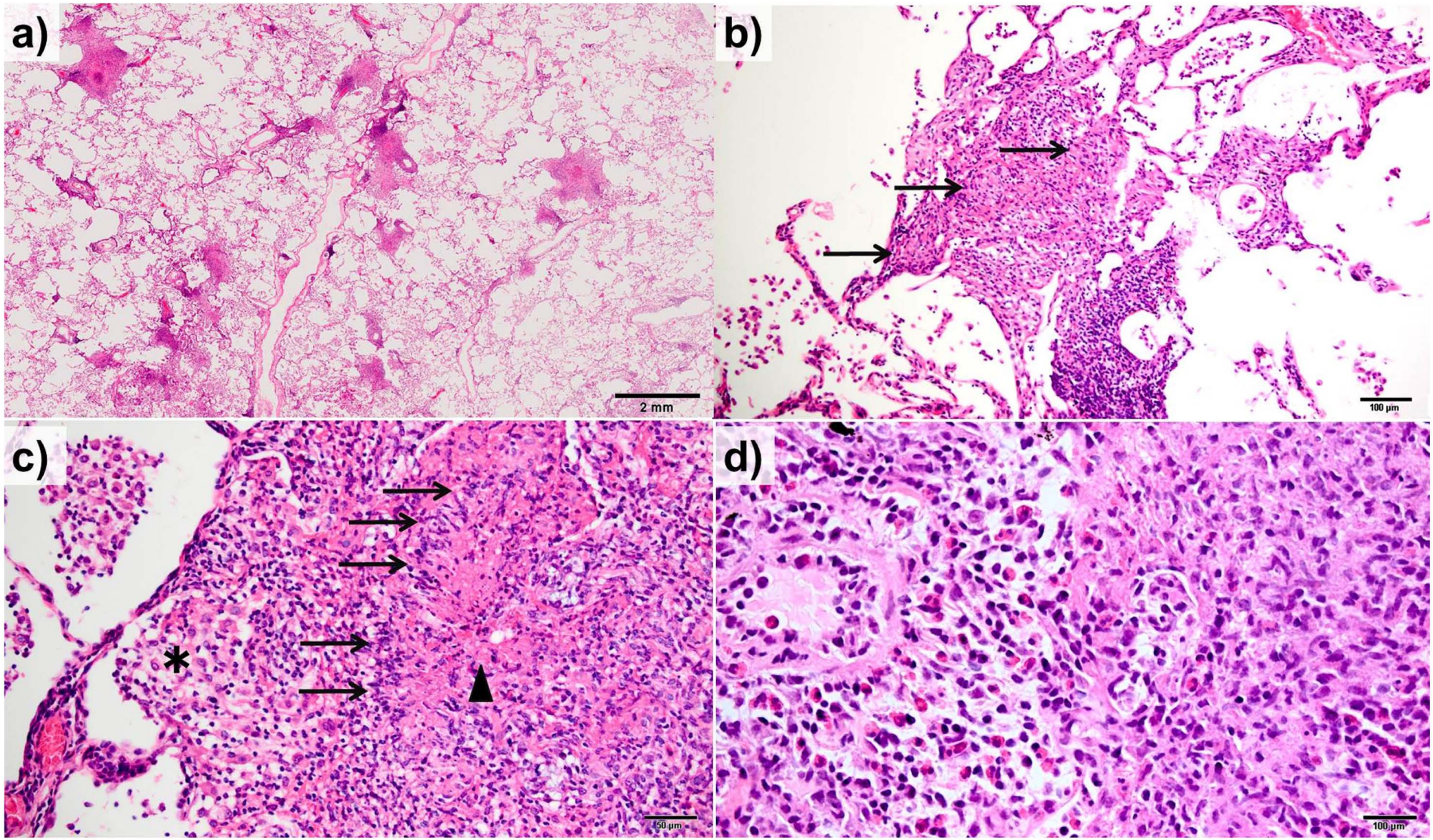

Table1 Summary of clinical data

| | case1 | case2 | case3 |
|---|---|---|---|
| sex | male | male | male |
| age at first admission | 75 | 65 | 70 |
| chief complaint | cough, sputum | dry cough | dry cough, dyspnea |
| smoking history | ex-smoker | ex-smoker | ex-smoker |
| BAL cell count | 5.0x10^5 | 3.3x10^5 | 5.8x10^5 |
| BAL: CD4/CD8 | 3.51 | 4.53 | 3.03 |
| KL-6 | 769 | 557 | 1819 |
| LDH | 233 | 369 | 249 |
| ATLA | positive | positive | positive |
| sIL-2R | 1780 | 1800 | 2040 |
| ANA/CVD specific antigen | – | RNP, SS-A, SS-B, Scl-70, Jo-1 | – |
| culture | – | – | – |
| treatment | low dose steroid | none | low dose erythromycin, inhaled steroid |
| prognosis | 5years/slowly growing | 9years/improved | 12years/improved |

Table2 Summary of radiological findings

| | case1 | case2 | case3 |
|---|---|---|---|
| Miliary centrilobular nodules | Yes | Yes | Yes |
| Pleural nodule | Yes | Yes | Yes |
| Interlobular septal thickening | Yes | Yes | Yes |
| Emphysema | No | Yes | No |
| Bronchiectasis | No | Yes | Yes |
| Ground glass opacity | No | No | No |
| Consolidation | No | No | No |

Table 3 Summary of pathological findings

| | case1 | case2 | case3 |
|---|---|---|---|
| Eosinophilia | ++ | ++ | + |
| Granuloma | + | + | + |
| Necrosis | + | + | – |
| Bronchiolitis | – | +/– | – |
| Airspace organization | + | + | ++ |
| Foamy macrophage | – | + | – |
| Tcell lymphocytosis | ++ | + | + |