

[ CASE REPORT ]

## Secondary Pulmonary Alveolar Proteinosis Associated with Primary Myelofibrosis and Ruxolitinib Treatment: An Autopsy Case

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### Abstract:

Pulmonary alveolar proteinosis (PAP) is an uncommon lung disorder characterized by the excessive accumulation of surfactant-derived lipoproteins in the pulmonary alveoli and terminal bronchiole. Secondary PAP associated with primary myelofibrosis (PMF) is extremely rare, and to our knowledge, no autopsy case has been reported. We herein report an autopsy case of secondary PAP occurring in a patient with PMF who was treated with the Janus kinase 1/2 inhibitor ruxolitinib. We confirmed a diagnosis of PAP with complications based on the pathological findings at the autopsy. Notably, this case might suggest an association between ruxolitinib treatment and PAP occurrence.

**Key words:** pulmonary alveolar proteinosis, primary myelofibrosis, autopsy, ruxolitinib

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### Introduction

Pulmonary alveolar proteinosis (PAP) is an uncommon lung disorder characterized by the excessive accumulation of surfactant-derived lipoproteins in the pulmonary alveoli and terminal bronchiole (1). PAP can be classified into three clinically distinct forms: autoimmune, hereditary, and secondary (2). Secondary PAP results from conditions involving functional impairment or reduced numbers of alveolar macrophages; the most frequent underlying disease is hematological malignancy (2).

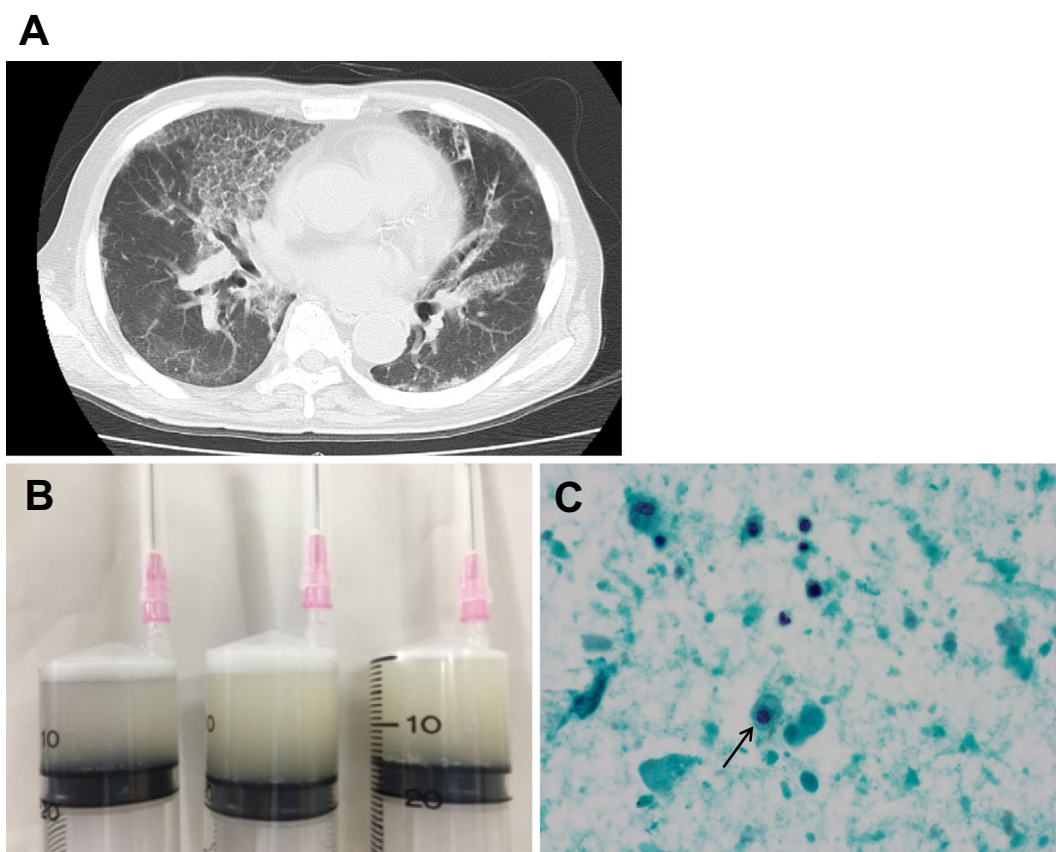
Primary myelofibrosis (PMF) is a myeloproliferative neoplasm associated with bone marrow fibrosis, pancytopenia, constitutional symptoms (e.g., night sweats, pruritus, weight loss, and a fever), hepatosplenomegaly, and extramedullary hematopoiesis (3). Ruxolitinib, a Janus kinase (JAK) 1/2 inhibitor, is generally administered to relieve PMF-related symptoms (4), and it is also approved for the treatment of polycythemia vera (PV). Because thrombocytopenia is a

dose-limiting toxicity, the initial dose of myelofibrosis is based on the platelet count, as follows: platelet count >200,000/ $\mu$ L: 20 mg twice daily and platelet count 100,000-200,000/ $\mu$ L: 15 mg twice daily. Common adverse events of ruxolitinib include hematological toxicity, diarrhea, and fatigue (4).

We herein report an autopsy case of secondary PAP occurring in a patient with PMF who had been treated with ruxolitinib. The patient also experienced complications of pneumocystis pneumonia (PCP) and *Mycobacterium Avium* Complex (MAC) infection. Secondary PAP associated with PMF is extremely rare, and to our knowledge, no autopsy case has been reported. In this case, the autopsy was diagnostically important because PAP and the underlying disease were confirmed by definitive pathological findings, which helped exclude other pulmonary diseases. Notably, there has been only one case report describing the onset of PAP during the administration of ruxolitinib, which occurred in a patient after hematopoietic stem cell transplantation administered for myelodysplastic syndrome (MDS) (5). Our case

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**Figure 1.** (A) Chest computed tomography on the day of admission showed bilateral ground glass opacities and intralobular and interlobular septal thickening (crazy-paving appearance). (B) The bronchoalveolar lavage fluid (BALF) showed a light milky appearance. (C) A Papanicolaou-stained cytologic smear of the BALF showed foamy macrophages (arrowhead) with a background of amorphous materials (magnification  $\times 600$ ).

suggests an association between ruxolitinib and the occurrence of PAP.

### Case Report

A 70-year-old man was referred to our hospital with appetite loss and general malaise. He had severe anemia and splenomegaly. A bone marrow biopsy showed bone marrow fibrosis, and he was diagnosed with PMF. A mutation analysis of *JAK2V617F* for this patient was negative. The *JAK2V617F* mutation is often found in patients with myeloproliferative disease, including 60-65% of patients with PMF, 95-97% with PV, and 60-65% with essential thrombocythemia (6). The efficacy of ruxolitinib is independent of this mutation (7). Seven years after he was diagnosed with PMF, he began outpatient treatment with ruxolitinib to ameliorate anemia and splenomegaly. Since starting ruxolitinib administration, he had not shown any respiratory symptoms. Two years later, he developed respiratory failure and a high fever that did not respond to oral antibiotics, and he was admitted to our hospital.

Computed tomography showed diffuse ground grass opacity in the bilateral lungs (crazy-paving pattern) (Fig. 1A). Laboratory data at the time of admission are shown in Ta-

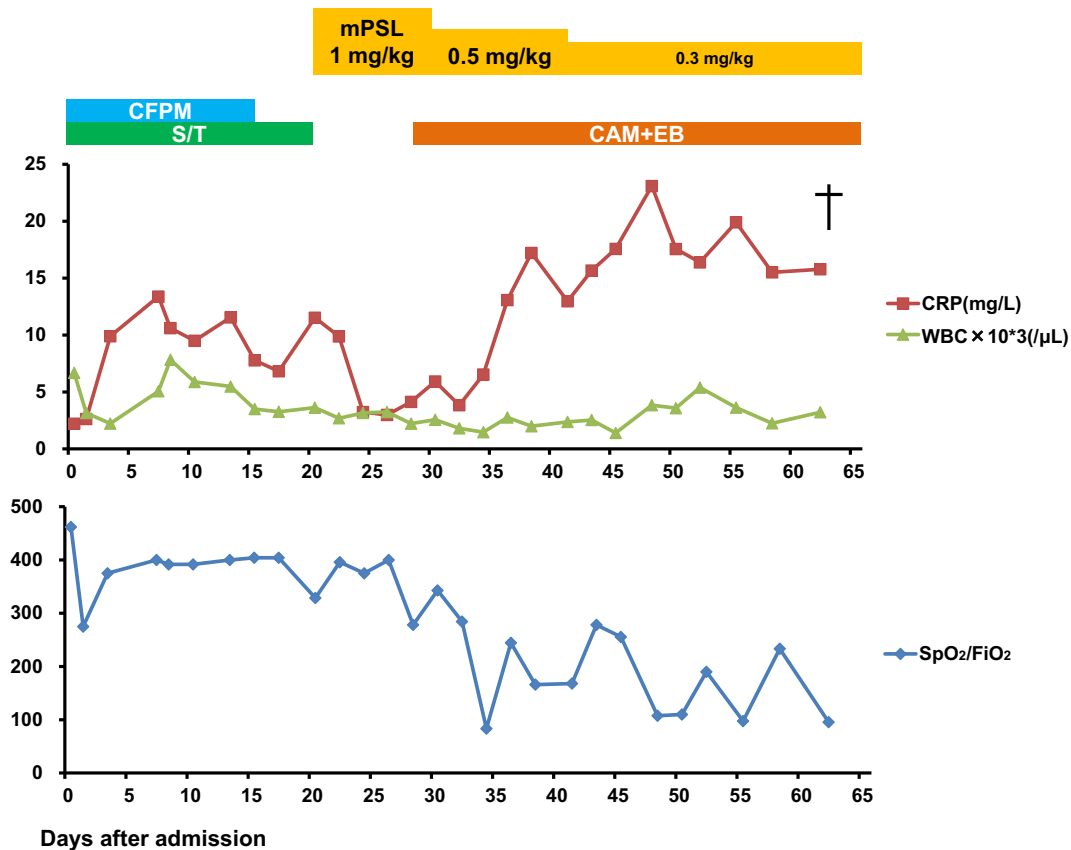
ble. In summary, C-reactive protein (CRP), lactate dehydrogenase, Krebs von den Lungen-6, and  $\beta$ -D-glucan levels were increased (shown in Bold). Hemoglobin, hematocrit, and platelet counts were decreased (shown in *Italic*). His white blood cell count and surfactant protein-D levels were within the normal range.

Bronchoscopy revealed milky bronchoalveolar lavage fluid (BALF), which showed foamy macrophages with a background of amorphous materials by Papanicolaou stain (Fig. 1B, C). The total cell number in the BALF was  $1.2 \times 10^6/\text{mL}$ . The proportions of macrophages, lymphocytes, neutrophils, eosinophils, and basophils were 11%, 9%, 21%, 59%, and 0%, respectively, indicating an elevation in the eosinophil proportion. The CD4/CD8 T lymphocyte ratio was 0.5. Polymerase chain reaction for *Pneumocystis jirovecii* in the BALF was positive. An enzyme-linked immunosorbent assay of the patient's serum was performed by the Department of Bioscience Medical Research Center, Niigata University Medical & Dental Hospital (Niigata, Japan), and it was found to be negative for anti-granulocyte macrophage colony-stimulating factor (GM-CSF) antibody (0.23  $\mu\text{g/mL}$ , cut-off value  $>1.0 \mu\text{g/mL}$ ).

He was diagnosed with secondary PAP based on the milky appearance of the BALF, which showed characteris-

**Table. Laboratory Data at Admission.**

	Patient's data	Cutoff value
C-reactive protein (CRP)	2.21 mg/dL	<0.15 mg/dL
Lactate dehydrogenase (LDH)	785 U/L	<222 U/L
Krebs von den Lungen-6 (KL-6)	1,835 U/mL	<500 U/mL
Surfactant protein-D (SP-D)	<17.2 ng/mL	<110 ng/mL
$\beta$ -D-glucan ( $\beta$ -DG)	66.1 pg/mL	<11 pg/mL
White blood cell (WBC)	$6.68 \times 10^3 / \mu\text{L}$	$<8.6 \times 10^3 / \mu\text{L}$
Hemoglobin (Hb)	6.1 g/dL	>13.7 g/dL
Hematocrit (Ht)	16.7 %	>40.7 %
Platelet (Plt)	$14.9 \times 10^4 / \mu\text{L}$	$>15.8 \times 10^4 / \mu\text{L}$



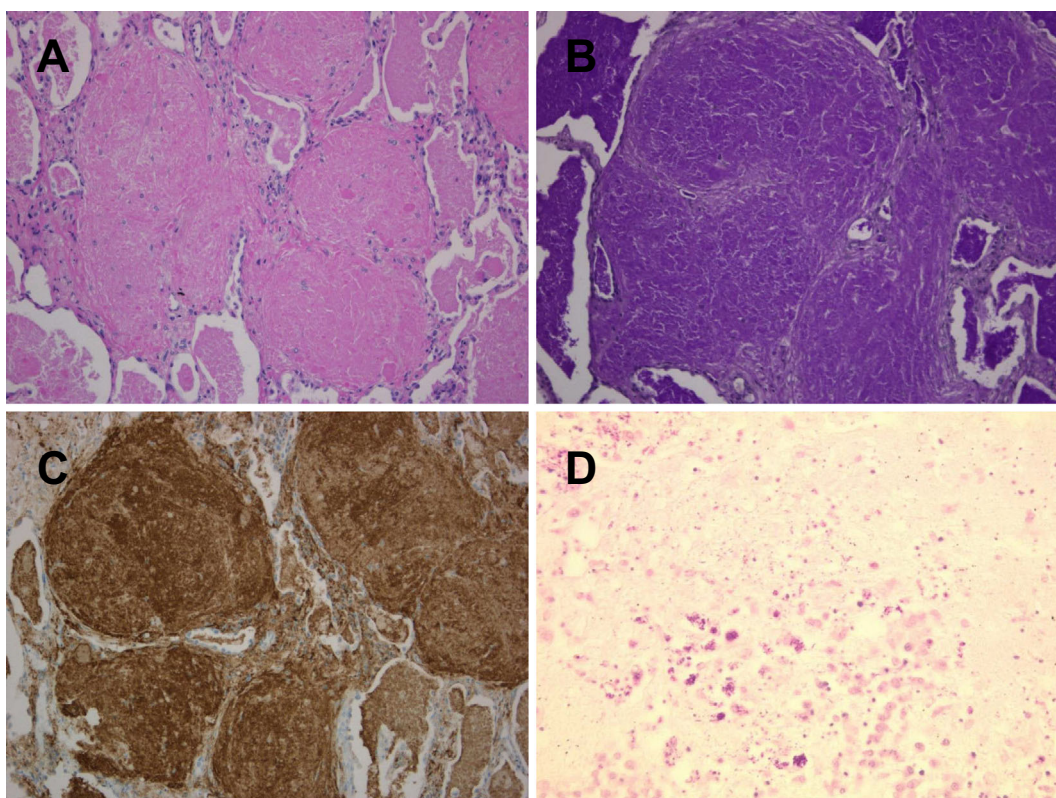
**Figure 2.** Clinical course shown by CRP, WBC and the SpO<sub>2</sub> to FiO<sub>2</sub> ratio. CRP: C-reactive protein, WBC: white blood cell, SpO<sub>2</sub>: saturation of percutaneous oxygen, FiO<sub>2</sub>: fraction of inspiratory oxygen, CFPM: cefepime, S/T: sulfamethoxazole and trimethoprim, CAM: clarithromycin, EB: ethambutol, mPSL: methylprednisolone

tics findings for a smear by Papanicolaou stain, his history of PMF, and anti-GM-CSF antibody negativity. He was also diagnosed with complication of PCP because of the high levels of  $\beta$ -D-glucan and *P. jirovecii* positivity.

He was treated by sulfamethoxazole and trimethoprim for PCP and cefepime for the bacterial infection, which may have caused complications. Ruxolitinib was discontinued because of active infection. During antibiotic treatment, the patient's temperature returned to normal, and the CRP and  $\beta$ -D-glucan levels decreased. However, antibiotics seemed to be ineffective, as the patient developed a second fever, and his CRP level increased three weeks after admission. Be-

cause of the increased eosinophil proportion in the BALF, we considered the possibility of complication of eosinophilic pneumonia and started methylprednisolone at 1 mg/kg. His temperature decreased again, and the CRP levels seemed to recover, but they became exacerbated again four weeks after admission. *Mycobacterium intracellulae* was then detected in the sputum and BALF collected at the admission, and the serum samples were positive for MAC antibody. He was diagnosed with complications of pulmonary MAC infection and started clarithromycin and ethambutol. However, his respiratory failure progressed, and he died 65 days after admission. The clinical course is shown in Fig. 2. With the pa-





**Figure 3.** Histopathological sections from the lung autopsy showed filling of the alveolar spaces with eosinophilic granular substances on Hematoxylin and Eosin staining (A); the cells were Periodic acid-Schiff (PAS) stain-positive (B) and surfactant A stain-positive (C) (magnification  $\times 200$ ). Ziehl-Neelsen staining indicated acid-fast bacteria and invasion of inflammatory cells (D) (magnification  $\times 400$ ). These findings were consistent with PAP compromised with MAC infection. PAP: pulmonary alveolar proteinosis, PAS: periodic acid-Schiff, MAC: mycobacterium avium complex

tient's family's consent, we performed an autopsy.

The autopsy showed that both lungs were filled with eosinophilic material. This eosinophilic material in the alveoli was positive for Periodic acid-Schiff and surfactant A stains. Ziehl-Neelsen stain indicated invasion of inflammatory cells and acid-fast bacteria in the lung, which suggested persistent MAC infection (Fig. 3). There was no abnormal invasion of eosinophils in the autopsied lung. There was also no increase in myeloblasts in the bone marrow. Based on these findings, the major cause of death was deemed to be progression of PAP and pulmonary MAC infection.

## Discussion

To our knowledge, this is the first autopsy case of secondary PAP associated with PMF. Although there have been many case reports of PAP associated with MDS, there has been only one other case report of PAP secondary to PMF, and an autopsy was not performed in that report (8). In the present case, the autopsy was diagnostically important because PAP and the complicated disease were confirmed by definitive pathological findings, which helped exclude other pulmonary diseases. We confirmed complications of pulmonary MAC infection and excluded complications of eosinophilic pneumonia based on the pathological findings ob-

tained at the autopsy.

Notably, in the present case, PAP occurred during administration of the JAK1/2 inhibitor ruxolitinib, which might suggest an association between ruxolitinib and PAP occurrence. Ruxolitinib is a generally effective treatment option for relieving PMF-related symptoms, such as appetite loss, dyspnea, fatigue, insomnia, and pain (4). There is only one other case report describing the onset of PAP during ruxolitinib administration in a patient after hematopoietic stem cell transplantation for MDS (5). The authors suggest that ruxolitinib may lead to the development of PAP by disrupting intracellular GM-CSF signaling, as the GM-CSF receptor is coupled with a JAK, and the first step of intracellular signaling in macrophages depends on JAK2 activation and STAT3 phosphorylation. However, to our knowledge, there are no reports showing a direct association between STAT3 inhibition and the occurrence of PAP. Hildebrandt et al. reported that reduced STAT5 phosphorylation in peripheral blood mononuclear cells and granulocytes was seen in six cases of juvenile PAP caused by CSF2RA mutations (9). In their report, the median age at the symptom onset was 3.5 years (9). In another report, mononuclear cells of mice treated with ruxolitinib showed a trend toward a reduced phosphor-STAT5 induction in response to *in vitro* GM-CSF stimulation, although this trend was not statistically signifi-

cant (10). In the present case, an onset of two years from the start of ruxolitinib treatment seems to be reasonable based on the immunological abnormality of PMF. Given these present and previous findings, we consider the mechanism of PAP development to be as follows: 1) PMF with a background of immunological abnormality tends to induce PAP, and 2) ruxolitinib administration affects the JAK/STAT pathway.

In addition, the etiology of secondary PAP has not been completely elucidated, a mouse study demonstrated that macrophage dysfunction had a pivotal role in the development of PAP (11), and ruxolitinib can inhibit macrophage activity and hemophagocytic lymphohistiocytosis in a mouse model (12, 13). In these reports, ruxolitinib inhibited STAT5 phosphorylation of macrophages and macrophage-stimulating cytokines, such as interferon- $\gamma$  and tumor necrosis factor- $\alpha$  (12, 13). Theoretically, it is possible that ruxolitinib affects the alveolar macrophage function and results in the development of PAP.

In the present case, the patient developed two opportunistic infections in the lung: PCP and pulmonary MAC infection. PAP patients are likely to develop opportunistic infections in the lung because of the dysfunction of lung macrophages. Furthermore, the underlying disease of this patient was myelofibrosis, and he was extremely immunocompromised and therefore prone to suffer from opportunistic infections. The major causes of death in secondary PAP patients complicated with MDS, which is a disease similar to PMF, are progression of PAP and infectious disease (14). The administration of ruxolitinib might also be related to opportunistic infections of the lung, as ruxolitinib has been reported to have an immunosuppressive effect, which can increase the risk of infection (15). Ruxolitinib is especially known to be a risk factor of pneumonia (16) and mycobacterial infection (17). Interestingly, a previously reported case of PAP associated with ruxolitinib was complicated with *M. abscessus* lung infection (5). To our knowledge, the accurate incidence of PCP and mycobacterial infection during ruxolitinib treatment has not been reported, but ruxolitinib suppresses the JAK1/2 signaling pathway, thereby affecting many cytokines and growth factors that are important for the immune function. Therefore, ruxolitinib may theoretically affect the development of these infections. Conversely, prednisolone was reported as a risk factor for infection in patients with autoimmune PAP (18). We started prednisolone because of the possibility of complication of eosinophilic pneumonia, and this may have worsened the pulmonary MAC infection.

In conclusion, we experienced a case of secondary PAP occurring in a patient with PMF who was being treated with ruxolitinib. The patient also developed PCP and pulmonary MAC infection. We confirmed the diagnosis of PAP and the complicated disease based on the pathological findings obtained at the autopsy. The JAK1/2 inhibitor ruxolitinib might be related to the development of PAP and subsequent opportunistic infection.

**The authors state that they have no Conflict of Interest (COI).**

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