Autoimmune Pulmonary Alveolar Proteinosis Diagnosed After Exposure to a Fire Extinguisher Containing Silica Powder: A Case Study

Takafumi Yorozuya1, Kimiyuki Ikeda1, Hirofumi Chiba1, Atsushi Saito1,2, Koji Kuronuma1, Hirotaka Nishikiori1, Satsuki Miyajima1, Mamoru Takahashi1, Takumi Yoshikawa1, Youhei Takahashi1, Tetsuya Taya1, Yuki Mori1, Yasuaki Umeda1, Mitsuo Otsuka1, Hiroshi Moriyama3 and Hiroki Takahashi1

Abstract:
We herein report a case of autoimmune pulmonary alveolar proteinosis (PAP) diagnosed after one-time exposure to silica powder. Owing to the misuse of a silica-containing fire extinguisher and the inhalation of large amounts of its powder, the patient experienced prolonged cough and visited our hospital. The findings of chest computed tomography and surgical lung biopsy specimens led to the diagnosis of PAP. Interestingly, the presence of anti-GM-CSF antibody was detected; therefore, both autoimmune characteristics and exposure to large amounts of silica may have caused the development of PAP in this patient. This case provides important insight into the mechanisms leading to the onset of PAP.

Key words: pulmonary alveolar proteinosis, Anti-GM-CSF antibody, dust, silica, fire extinguisher, elemental analysis

Background
Pulmonary alveolar proteinosis (PAP), first described by Rosen et al. in 1958 (1), is a rare disease characterized by the accumulation of surfactant-derived lipids and proteins in the alveolar spaces due to a disruption of surfactant homeostasis. Based on the etiology, it can be classified into three clinical forms, namely autoimmune, secondary, and congenital. In autoimmune PAP (APAP), neutralizing autoantibody against granulocyte/macrophage colony-stimulating factor (GM-CSF) causes the dysfunction of the alveolar macrophages and neutrophils, thereby resulting in the development of APAP (2, 3).

An international epidemiological survey conducted by Inoue et al. in 2008 revealed that >90% of patients with PAP were diagnosed with APAP, among whom 23% had histories of dust inhalation (4). However, the mechanism of the underlying development of APAP, particularly by exposure to dust, remains unknown.

We herein report the case of a patient with APAP who was diagnosed after one-time exposure to large amounts of silica powder from a fire extinguisher and discuss the effect of dust exposure on the development of this disease.

Case Report
A 76-year-old Japanese woman misused a fire extinguisher and continued to discharge its powder for about 10 sec in a room without a window, thereby inhaling a large amount of dust in early October 2015. She had been asymptomatic for 2 weeks after the incident; however, she had been coughing since mid-October. She visited a local outpatient clinic in late October; chest radiography showed a dif-
fuse infiltrate shadow (Supplementary Fig. S1). She showed no improvement after the administration of cough suppressants by the clinic, and thereafter visited another clinic in early December. Chest computed tomography (CT) showed diffuse infiltrate shadows in the bilateral lung fields, and she was hospitalized at the Sapporo Medical University Hospital in late December.

She had been working in the seafood processing industry since 18 years of age, and had no history of smoking and also no occupational or environmental exposure to dust. While previously she had WPW syndrome and dyslipidemia, there was no history of hematological diseases, autoimmune diseases, inflammatory bowel disease, or pulmonary infection. On examinations before the incident, chest radiographs showed no abnormalities in 2006, 2011, and 2012; her lactate dehydrogenase (LDH) level was within the normal range at 227 U/L [cutoff (CO) <240] in 2007.

Laboratory evaluation at the time of hospitalization revealed that her LDH, surfactant protein (SP)-A, and Krebs von den Lungen-6 (KL-6) levels were elevated at 294 U/L (CO <240), 77.9 ng/mL (CO <43.8), and 1,709 U/mL (CO <500), respectively. However, her white blood cell (WBC) count, C-reactive protein (CRP), and SP-D levels were within the normal range at 6.1x10³/μL (CO <9.8x10³), 0.10 mg/dL (CO <0.30), and 86.9 ng/mL (CO <110), respectively.

A respiratory physiological examination revealed a gas exchange disturbance. An arterial blood gas analysis showed the partial pressure of oxygen (PaO₂) to have decreased (62.9 Torr), while the alveolar arterial oxygen gradient (A-aDO₂) was elevated (39.2 Torr). Additionally, the minimum oxygen saturation in the 6-min walk test was decreased (89%). Pulmonary function tests showed the diffusion capacity for carbon monoxide (DLCO) to have decreased (68.6%) and the vital capacity (VC) remained (120.0%).

A radiological examination of the chest showed diffuse infiltrate shadows (Fig. 1A), and high-resolution CT showed multifocal ground glass opacities in the bilateral lower lobe dominance (Fig. 1B). Owing to the characteristic geographic distribution, intralobular and interlobular septa thickening, and crazy-paving appearance, PAP was suspected.

Bronchoalveolar lavage (BAL) was performed on hospital day 2 from the right B₁, and a recovery rate of 43.3% was obtained. Her BAL fluid (BALF) was colorless and transparent; it was not characteristic of PAP. A BALF evaluation showed the total cell number and the cell concentration to have decreased to 0.416x10³ and 0.640x10³/mL, respectively. The differential WBC count for macrophage, lymphocyte, neutrophil, eosinophil, and basophil was 77.8%, 19.6%, 2.6%, 0.0%, and 0.0%, respectively, showing a slight elevation of lymphocyte proportion. Among the T lymphocytes, the CD4/CD8 ratio was 1.8. The levels of SP-A, SP-D, and KL-6 in BALF were 1,665, 550 ng/mL, and 519 U/mL, respectively. Finally, no bacteria were detected in BALF.

A transbronchial lung biopsy was performed on the patient on hospital day 3, and samples were collected from the right S₁, S₂, and S₃; small amount of granular substances was present in the alveolar spaces, however, these findings did not enable us to make a definite diagnosis. Therefore, a surgical lung biopsy was performed in late January 2016 from the left S₁, S₂, and S₃. A histopathological evaluation revealed the alveolar spaces to be filled with anti-SP-A antibody (PE10)/periodic acid-Schiff (PAS)-positive eosinophilic granular substances and there were some foamy macrophages locally (Fig. 2), and these findings were consistent with the characteristics of PAP. An evaluation using a polarizing microscope revealed that hyaline-like structure sug-
sugesting of silicotic nodules were not present. In addition, there were no findings indicating any another disease such as lung injury.

Interestingly, the serum level of anti-GM-CSF antibody at her first visit to our hospital was found to be positive at 15.0 μg/mL. Based on these results, she was diagnosed with APAP which may have been caused by her autoimmune characteristics and her exposure to large amounts of dust.

We speculated that she would recover without any therapeutic intervention, such as whole lung lavage or GM-CSF therapy, because the effect of exposure to dust would be temporary. Therefore, we decided to follow up the patient without any specific treatment. Although her condition remained unchanged at first, infiltrate shadows on chest images seemed to improve in mid-March and then completely disappeared in late May. The serum biomarker levels at each time-point are summarized in Table. In addition, she was able to return to her normal work from April 2016 and did not change her living environment.

An elemental analysis was performed on lung tissue

Figure 2. Histopathological sections of a surgical lung biopsy show the filling of the alveolar spaces with eosinophilic granular substances with (A, B) Hematoxylin and Eosin staining (A, magnification ×100; B, ×200), which were (C) PE 10 and (D) PAS positive (magnification ×100). These finding were consistent with PAP. PAP: pulmonary alveolar proteinosis, PAS: periodic acid-Schiff, PE 10: anti-SP-A antibody

Table. Blood Biomarkers at Each Time-point.

<table>
<thead>
<tr>
<th></th>
<th>Late May 2007</th>
<th>Late Oct 2015</th>
<th>Late Dec 2015</th>
<th>Mid Mar 2016</th>
<th>Late May 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (/μL)</td>
<td>3,600</td>
<td>6,170</td>
<td>4,800</td>
<td>5,200</td>
<td>4,500</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>227</td>
<td>288</td>
<td>292</td>
<td>223</td>
<td>209</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.29</td>
<td>0.59</td>
<td>0.14</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>SP-A (ng/mL)</td>
<td>NA</td>
<td>NA</td>
<td>77.9</td>
<td>57.7</td>
<td>41.5</td>
</tr>
<tr>
<td>SP-D (ng/mL)</td>
<td>NA</td>
<td>NA</td>
<td>86.9</td>
<td>89.1</td>
<td>56.4</td>
</tr>
<tr>
<td>KL-6 (U/mL)</td>
<td>NA</td>
<td>NA</td>
<td>1,709</td>
<td>2,462</td>
<td>709</td>
</tr>
<tr>
<td>GM-CSF Ab (μg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>15.0</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Figure 3. An elemental analysis of lung tissue specimens using an Electron Probe Micro Analyzer. (A) The deposition of silicon (Si) was detected in the alveolar spaces, and (B) Si is also observed in a large proportion around the bronchioles. The deposition of Si is shown in yellow.


Element | Weight (%) |
---------|------------|
Si       | 28.466     |
O        | 26.382     |
Fe       | 21.734     |
Al       | 8.641      |
K        | 4.143      |
P        | 2.460      |
Ca       | 2.170      |
Na       | 1.737      |
S        | 1.395      |
Zn       | 1.079      |
Mg       | 0.912      |
Ti       | 0.664      |
Cl       | 0.216      |
**Total** | **100.000** |

Discussion

Our patient would have been diagnosed as secondary PAP (SPAP) using the previous disease classification (i.e., before anti-GM-CSF antibody was measurable) because it had been diagnosed after dust inhalation from a fire extinguisher. SPAP has been reported to be caused by hematological diseases, including myelodysplastic syndrome, lymphoma and leukemia, Behçet’s disease, autoimmune diseases, and mycobacterium infections (7) and the inhalation of dust, including silica, aluminum (Al), and titanium (8-11). However, because our patient was also positive for anti-GM-CSF antibody, she was thus diagnosed with APAP rather than SPAP based on the diagnostic flow chart of Inoue et al. (4).

Another report of dust-related PAP with anti-GM-CSF antibody discussed a case caused by occupational exposure to indium-tin oxide for 4 months (12). Additionally, anti-GM-CSF antibodies were reported to be positive in many patients with pneumoconiosis who merged PAP (13). However, there are no reports on PAP with anti-GM-CSF antibody detected after one-time exposure to dust. It is believed that the one-time exposure of silica does not always result in PAP. Thus, she might have had some onset factor, although she was asymptomatic before dust exposure from the fire extinguisher.
guisher. Taken together, she might have potential characteristi-
cic of APAP. Unfortunately, the serum of the patient prior to
the dust exposure was unavailable; nevertheless, this case
can be highly significant for understanding the mechanism
underlying the development of APAP.

During APAP, anti-GM-CSF antibody causes a deteriora-
tion of the functions of alveolar macrophages, such as their
migratory and phagocytic ability, and, thereby, results in a
decrease in the surfactant elimination capacity (2, 3). More-
over, a report has suggested that in pulmonary alveolar mi-
crotheliasis, the presence of calcium phosphate stones causes
surfactant accumulation via the dysfunction of alveolar
macrophages (14). Considering these studies, it is possible
that the deteriorated alveolar macrophages reach their elimi-
nation capacity limit upon dust exposure. Consistent with
this, in the large cohort of 223 APAP patients studied in
Inoue et al., 23% of the patients had a history of dust inha-
lation (4). Our case showed that large amounts of dust expo-
sure, even just one-time, may cause PAP.

Although many cases of SPAP induced by dust inhalation
have been reported previously, most of them were reported
before anti-GM-CSF antibody became measurable. In Japan,
one case of dust-related PAP without anti-GM-CSF antibody
was reported after the autoantibody became measurable (15).
However, even in this case, the serum level of anti-GM-CSF
antibodies exceeded the cutoff level at 1 year after exposure;
in addition, *in vitro* studies revealed that the serum from this
patient had an inhibitory effect on GM-CSF signaling (15).
These findings suggest that the measurement of anti-GM-
CSF antibody is necessary even in cases of dust-related PAP.

We found just one case of PAP induced by the contents
of a fire extinguisher, which was reported by Kim et
al. (16). The patient in this case had been working at a facil-
ity manufacturing gas-type fire extinguishers using hydro-
fluorocarbon (HF) and developed PAP after repeated expo-
sure to fire extinguisher spray. In our case, the main compo-
nents of the fire extinguisher were silica, ammonium phos-
phate, and ammonium sulfate, but not HF. Therefore, our
case is the first report of PAP diagnosed after the inhalation
of a powder-type fire extinguisher. Patients with PAP or pa-
tients with anti-GM-CSF antibody-positive should therefore
be extremely careful while using such fire extinguishers.

In our case, the ratio of elements detected by EPMA was
high in the order of Si, O, iron (Fe), and Al (Fig. 4). Adachi
et al. showed that the intratracheal exposure of rats to silica
increased the surfactant synthesis and secretion from the al-
veolar type II cells and impaired the surfactant removal sys-
tem (17). Regarding Fe, it has been reported that patients
with PAP have a disorder of iron homeostasis, and the
hemosiderin-laden macrophages in their lung tissue tend to
contain large amounts of iron (18, 19).

Crystalline silica and silicotic nodules are observed in the
lungs of patients who repeatedly inhale silica, and such pa-
tients are diagnosed with chronic silicosis. We did not find
these pathological findings in our patient using a polarizing
microscope. Although conditions with a rapid onset are
called acute silicosis or silicoproteinosis, patients with expo-
sure shorter than 1 year are extremely rare. To our knowl-
edge, the shortest exposure period in case reports of PAP in-
duced by dust exposure, irrespective of silica, is several
weeks (20, 21). Although Xiao et al. reported four cases of
dust-related PAP without anti-GM-CSF antibody, these pa-
tients were diagnosed with silicoproteinosis, which is clin-
cal and radiologically different from typical PAP, and had a
very poor prognosis (22).

Some patients with PAP in whom BALF was colorless
and transparent, similar to that in our patient, have been re-
ported (23-35). They had milder symptoms and the extent of
shadows on the chest images was not so intense. Similarly,
in our patient, the extent of a shadow observed on right S4
where BAL was performed was mild, which may have re-
sulted in the BALF findings.

The levels of SP-A, SP-D, and KL-6 in sera and BALF in
this patient were significantly lower than those in patients
with PAP reported previously (26-28). Although BALF can
be explained by the above paragraph, the sera remain un-
known. One possible explanation is: due to the inhalation of
fire extinguisher powder, the disease developed relatively
rapidly; as a result, production of SP-A, SP-D, and KL-6
from alveolar type II cells were insufficient, and leakage of
them into the blood was difficult.

It is presumed that silica from the fire extinguisher still
remained in the lung due to the lack of any removal mecha-
nism. Nonetheless, PAP in this patient recovered without
therapeutic intervention. There is a possibility that the
phagocytic capacity could have been complemented by
macrophages newly recruited to the alveolus. If so, the anti-
GM-CSF antibody might not have inhibited the differentia-
tion and proliferation of alveolar macrophages completely
in this patient. Similarly, in the cohort study by Inoue et al.,
28.2% of asymptomatic APAP participants at enrollment had
experienced a spontaneous improvement since the onset (4).

In our case, we speculated that dust exposure had led to
the development of PAP in a patient with autoimmune char-
acteristics. Conversely, reports have shown that dust expo-
sure induces anti-GM-CSF antibody as an epipheno-
non (29, 30). In our case, the serum of the patient prior to
dust exposure was unavailable, and, thus, the limitation of
determining when anti-GM-CSF antibodies were developed
remains. In the future, the further accumulation of such
cases is needed. In conclusion, our case study provides sig-
nificant insight into the effect of dust exposure on the de-
velopment of APAP.

**Informed Consent**

Written informed consent for the publication of the clinical
details and images was obtained from the patient.

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

**Acknowledgement**

The authors would like to thank Niigata University for the
EPMA of the specimens. The work of the EPMA was supported by JSPS KAKENHI Grant Number JP17K09635.

References


