

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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## **Supplementary Appendix**

### **PARTICIPANTS**

#### **PULMONARY ALVEOLAR PROTEINOSIS (PAP) PATIENTS**

All 12 PAP patients included in the study had the typical clinical, physiological, and radiographic features of the disease at the time of evaluation. The diagnosis of PAP was based on clinical and radiographic findings and an open lung biopsy (Patients 2, 3, 6, 7, 9, 12), transbronchial lung biopsy (Patients 4, 5, 8, 10) or cytological analysis of bronchoalveolar lavage cells and fluid (Patients 1, 11). Two clinical presentations predominated: (1) dyspnea of insidious onset (Patients 4, 5, 7, 8) or (2) a persistently abnormal chest radiograph suggestive of pneumonia but without response to antibiotics (Patients 1-3, 6, 10-12). The mean ( $\pm$  SE) duration of symptoms at the time of study for pediatric and adult cases was  $22.7 \pm 2.2$  and  $46.7 \pm 9.0$  months, respectively, which was not significantly different ( $P = 0.167$ ). The mean ( $\pm$  SE) age at diagnosis in pediatric ( $<18$  years) and adult cases was  $13.7 \pm 0.9$  and  $43.3 \pm 4.3$  years, respectively. The mean ( $\pm$  SE) anti-GM-CSF autoantibody titer in PAP patients was  $324.3 \pm 66.0$   $\mu\text{g/ml}$  and was similar in adult and pediatric cases ( $365 \pm 74$  and  $202 \pm 141$   $\mu\text{g/ml}$ , respectively;  $P = 0.308$ ).

Patient 1 is a Caucasian man who presented at age 37 to another hospital with a new-onset generalized seizure. Magnetic resonance scanning revealed cavitory lesions of the cerebrum; anticonvulsants and broad spectrum antibiotics were started and he was transferred to our center for further evaluation and treatment. Stereotatic brain biopsy confirmed the presence of cerebral abscesses and *Staphylococcus epidermidis* grew from a culture of the cerebral spinal fluid. He was a previously healthy construction worker and had a 21 pack-year history of

cigarette smoking but no other pulmonary exposures. He denied respiratory or other symptoms, however, the chest radiograph on admission revealed diffuse pulmonary ground glass opacities. Bronchoscopy with bronchoalveolar lavage (BAL) was nondiagnostic. Antibiotic therapy was continued for eight weeks, during which time dyspnea developed insidiously, worsened progressively and was eventually accompanied by a non-productive cough. Five months after initial presentation, bronchoscopy was repeated and a diagnosis of PAP was established based on the history, computed tomography (CT) of the chest and cytological analysis of BAL cells and fluid. He underwent whole lung lavage (WLL) therapy, which resulted in significant clinical improvement. Forty months later the patient again presented to his primary physician with progressive dyspnea, digital clubbing, and polycythemia. He was referred to our center 46 months after onset of symptoms and enrolled in the study. WLL therapy was performed and resulted in significant clinical improvement.

Patient 2 is an African-American boy who was admitted at age 11 to another hospital with right upper lobe pneumonia and pulmonary abscess. He was a previously healthy, athletic student with no history of smoking or pulmonary exposures. Antibiotic therapy resulted in clinical improvement and resolution of the abscess. Twelve months later, he presented with dyspnea and fever and a chest radiograph revealed diffuse pulmonary infiltrates. Several courses of oral antibiotics were administered without improvement and dyspnea, exercise intolerance and cough worsened. Four months later, CT of the chest revealed diffuse ground glass opacities and patchy consolidation; bronchoscopy revealed acute airway inflammation, and *Haemophilus influenzae* grew from a culture of the BAL fluid. Three months later, an open lung biopsy established the diagnosis of PAP. The patient was referred to our center 20 months after onset of symptoms and enrolled in the study. WLL therapy was performed and resulted in significant clinical improvement.

Patient 3 is a Caucasian girl who presented at age 14 to another hospital with dyspnea and exercise intolerance. She was a previously healthy student except for a history of “a spot of pneumonia” on chest radiograph taken four years earlier, with no history of smoking or pulmonary exposures. The chest radiograph revealed diffuse pulmonary interstitial infiltrates. Pulmonary function testing revealed moderate restrictive impairment and hypoxemia was treated with oxygen therapy. Bronchoscopy with cytological analysis of the BAL revealed neutrophilic inflammation and eosinophilic, Periodic Acid Schiff-positive material. *Influenza B* grew from a culture of the BAL fluid. Serial CT of the chest over the next two months revealed persistent bilateral ground glass opacities and an open lung biopsy established the diagnosis of PAP. She was referred to our center and underwent WLL therapy, which resulted in clinical improvement. WLL was performed six times before she enrolled in the study 21 months after onset of symptoms.

Patient 4 is a Caucasian woman who presented at age 23 to her primary care physician with abdominal pain. She was a previously healthy counselor, except for mild asthma, exercised regularly, and had no history of smoking or other pulmonary exposures. Erosive gastritis was diagnosed by endoscopy but was unresponsive to medical management. Cholecystectomy was performed without improvement. Ten months later, dyspnea and pleuritic chest pain developed and diffuse pulmonary infiltrates were noted incidentally on rostral sections of a CT of the abdomen, performed for evaluation of persistent epigastric pain. Transbronchial biopsy established the diagnosis of PAP. She was referred to our center 18 months after onset of symptoms and enrolled in the study. WLL therapy was performed and resulted in significant clinical improvement.

Patient 5 is a Caucasian girl who presented at age 13 to her primary physician with dyspnea on exertion. She was a previously healthy student with no history of smoking whose only known

potential pulmonary exposure was to mold in damp areas of the home. Albuterol and fluticasone propionate/salmeterol were administered for a presumptive diagnosis of asthma without clinical benefit. Nineteen months later, a radiograph and CT of the chest revealed bilateral opacifications and a diagnosis of bronchial pneumonia was entertained. Antibiotic therapy appeared initially to result in symptomatic improvement, however, dyspnea progressed unaccompanied by fever, cough or other symptoms. Five months later, CT of the chest revealed persistent diffuse infiltrates and a transbronchial biopsy established the diagnosis of PAP. She was referred to our center 27 months after onset of symptoms and enrolled in the study. WLL therapy was performed and resulted in significant clinical improvement.

Patient 6 is a Caucasian man who presented at age 32 to his primary physician with dyspnea, chills and fever. He was a restaurant manager and had a 5 pack-year history of cigarette smoking but no other pulmonary exposures. Several courses of oral antibiotics were administered without improvement. One month later, he was admitted to another hospital with severe dyspnea and cyanosis. A radiogram and CT of the chest revealed diffuse pulmonary infiltrates and an open lung biopsy established the diagnosis of PAP. He was referred to our center for WLL therapy, which resulted in significant improvement and was repeated annually. Forty two months after onset of symptoms, he was enrolled in the study.

Patient 7 is a Caucasian man who presented at age 51 to his primary physician with progressive dyspnea of insidious onset, cough and headaches. He was a banker with no history of smoking or other pulmonary exposures. A radiograph and CT of the chest revealed diffuse pulmonary opacities and an open lung biopsy established the diagnosis of PAP. He was referred to our center 13 months after onset of symptoms, enrolled in the study and underwent WLL, which resulted in clinical improvement. *Mycobacterium Avium Intracellulare* complex (MAC) grew from a culture of the WLL fluid and he was treated with antibiotics.

Patient 8 is a Caucasian woman who presented at age 39 to her primary physician with a two and one half year history of dyspnea of insidious onset, unaccompanied by other symptoms. She was an airline flight attendant and had smoked 1 pack of cigarettes for 20 years but had no other pulmonary exposures. A radiograph and CT of the chest revealed diffuse pulmonary opacities and a transbronchial biopsy established the diagnosis of PAP. She was referred to our center 35 months after onset of symptoms and enrolled in the study. WLL therapy was performed and resulted in significant clinical improvement.

Patient 9 is a Caucasian woman who was noted incidentally at the age of 36 to have diffuse bilateral pulmonary infiltrates on a routine chest radiogram, unaccompanied by respiratory symptoms. Bronchoscopy and transbronchial biopsy were nondiagnostic. She was a beautician and an emergency medical technician and had smoked two packs of cigarettes per day for multiple years but had no other pulmonary exposures. Dyspnea developed insidiously thereafter, progressed and three years later was accompanied by a cough productive of yellow-brown sputum prompting admission to another hospital. CT of the chest revealed dense bilateral interstitial infiltrates. Intravenous antibiotics and steroids were administered without clinical improvement. The hospital course was complicated by cough-induced pneumothorax, requiring thoracostomy tube placement, and persistent broncho-pleural fistula. Video-assisted thoroscopy with pleurodesis and oversewing of the pleura were performed to close the thoracostomy tube site; biopsies of the lung and chest wall established the diagnosis of PAP and presence of chest wall microabscesses, respectively. *Nocardia* and coagulase negative *Staphylococcus* grew from culture of the chest wall biopsy. Placement of a Heimlich valve resulted in resolution of the pneumothorax and she underwent WLL therapy, which resulted in clinical improvement. Seventy seven months after incidental identification of diffuse pulmonary infiltrates, she was enrolled in the study.

Patient 10 is a Caucasian man who presented at age 42 to his primary physician with acute onset of fever, cough, dyspnea and a skin rash. Pneumonia was diagnosed and antibiotics were administered, resulting in symptomatic improvement. He worked as a soil scientist but had no history of smoking or other pulmonary exposures. Over the following year, dyspnea, fatigue, and blurred vision developed. Fourteen months later, a transbronchial biopsy established the diagnosis of PAP. Two months later, GM-CSF therapy (250 µg/day) was administered by daily subcutaneous injection but was discontinued after one month due to worsening dyspnea. Oxygen therapy was initiated. Two months later, he underwent WLL therapy, which resulted in clinical improvement. One month later, WLL therapy was repeated, with minimal improvement. MAC grew from a culture of the WLL fluid and he was treated with antibiotics. One month later, WLL therapy was repeated, with clinical improvement. *Nocardia* grew from a culture of the WLL fluid and antibiotics were administered. WLL therapy was repeated at one to two month intervals thereafter for the next three years. Fifty seven months after initial presentation, GM-CSF therapy was restarted at a dose of 250 µg/day by subcutaneous injection with dose escalated to 1300 µg/day over seven months, but was discontinued when he developed hypertension and biopsy-proven membranous nephropathy. Sixty nine months after onset of symptoms, he was enrolled in the study.

Patient 11 is a Caucasian man who presented at age 60 at another facility with dyspnea. The chest radiograph was abnormal and he was treated with antibiotics with apparent clinical improvement. Eleven months later, he sought medical attention for persistent dyspnea after moving to an altitude of approximately 1,500 meters. He had a history of smoking and had worked in a research laboratory where he had been exposed to various chemicals and as a truck driver, transporting ammonia, sulfur, sulfates, nitric acid, fluorine and other chemicals.

Sixteen months later, a CT of the chest revealed diffuse pulmonary infiltrates. Fifteen months later, bronchoscopy was performed and MAC grew from a culture of the BAL fluid. Ciprofloxacin and Biaxin were administered for twenty months without resolution of the pulmonary infiltrates, at which time bronchoscopy with cytologic evaluation of the BAL fluid established the diagnosis of PAP. He underwent WLL therapy, which resulted in clinical improvement. WLL therapy was repeated three times prior to enrolling in the study 91 months after onset of symptoms.

Patient 12 is a Caucasian woman who presented to her primary physician at age 57 with dyspnea of insidious onset. The chest radiograph was abnormal and she was treated with oral antibiotics without improvement. She was a nurse and office assistant and smoked briefly in college but had no other pulmonary exposures. Eight months after presentation, progressive dyspnea and persistent radiographic abnormalities prompted bronchoscopic evaluation. *Mycobacterium tuberculosis* grew from a culture of the BAL fluid and she received isoniazid, rifampin and pyrazinamide. Four months later, an open lung biopsy established the diagnosis of PAP. Dyspnea progressed and she was referred to our center 29 months after onset of symptoms and enrolled in the study. WLL therapy was performed and resulted in significant clinical improvement.

#### **HEALTHY INDIVIDUALS**

Volunteers were enrolled in the study as healthy controls. This group included 48 females, 13 males, and had a mean ( $\pm$ SE) age of  $29.2 \pm 0.7$  years. All were disease-free, healthy individuals without a history of major illness and all were symptom-free at the time of enrollment in the study. None were current smokers. The upper limit of the confidence interval (ninety-ninth percentile) for the serum anti-GM-CSF antibody titer in this group was 3.52  $\mu$ g/ml.

**CYSTIC FIBROSIS PATIENTS**

Patients with cystic fibrosis were enrolled in the study as chronic disease controls. This group included 5 females, 1 male, and had a mean ( $\pm$ SE) age of  $24.3 \pm 1.4$  years. All were homozygous for  $\Delta F^{508}$  mutations in the cystic fibrosis transmembrane conductance regulator gene and had the typical clinical, radiographic and physiologic features of the disease. None were current smokers. The anti-GM-CSF antibody titer in this group was  $2.8 \pm 1.7$   $\mu$ g/ml.

**END STAGE LIVER DISEASE PATIENTS**

Patients with end stage liver disease were also enrolled in the study as chronic disease controls. This group included 3 females, 3 males, and had a mean ( $\pm$ SE) age of  $56.3 \pm 5.3$  years. All had cirrhosis, the underlying etiology of which included hepatitis C in two, non-alcoholic fatty liver in one, primary sclerosing cholangitis in one; cirrhosis was cryptogenic in two. All were undergoing periodic paracentesis. The anti-GM-CSF antibody titer in this group was  $3.4 \pm 1.8$   $\mu$ g/ml.