Characteristics of a Large Cohort of Patients with Autoimmune Pulmonary Alveolar Proteinosis in Japan

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Rationale: Acquired pulmonary alveolar proteinosis (PAP) is a syndrome characterized by pulmonary surfactant accumulation occurring in association with granulocyte/macrophage colony-stimulating factor autoantibodies (autoimmune PAP) or as a consequence of another disease (secondary PAP). Because PAP is rare, prior reports were based on limited patient numbers or a synthesis of historical data.

Objectives: To describe the epidemiologic, clinical, physiologic, and laboratory features of autoimmune PAP in a large, contemporaneous cohort of patients with PAP.

Methods: Over 6 years, 248 patients with PAP were enrolled in a Japanese national registry, including 223 with autoimmune PAP.

Measurements and Main Results: Autoimmune PAP represented 89.9% of cases and had a minimum incidence and prevalence of 0.49 and 6.2 per million, respectively. The male to female ratio was 2.1:1, and the median age at diagnosis was 51 years. A history of smoking occurred in 56%, and dust exposure occurred in 23%; instances of familial onset did not occur. Dyspnea was the most common presenting symptom, occurring in 54.3%. Importantly, 31.8% of patients were asymptomatic and were identified by health screening. Intercurrent illnesses, including infections, were infrequent. A disease severity score reflecting the presence of symptoms and degree of hypoxemia correlated well with carbon monoxide diffusing capacity and serum biomarkers, less well with pulmonary function, and not with granulocyte/macrophage colony-stimulating factor autoantibody levels or duration of disease.

Conclusions: Autoimmune PAP had an incidence and prevalence higher than previously reported and was not strongly linked to smoking, occupational exposure, or other illnesses. The disease severity score and biomarkers provide novel and potentially useful outcome measures in PAP.

Keywords: epidemiology; serum biomarkers; disease severity score; granulocyte/macrophage colony-stimulating factor; autoantibody

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Acquired pulmonary alveolar proteinosis (PAP) is a syndrome characterized by pulmonary surfactant accumulation occurring in association with granulocyte/macrophage colony-stimulating factor autoantibodies (autoimmune PAP) or as a consequence of another disease (secondary PAP). Because PAP is rare, prior reports were based on limited patient numbers or a synthesis of historical data.

What This Study Adds to the Field

Autoimmune PAP had an incidence and prevalence higher than previously reported and was not strongly linked to smoking, occupational exposure, or other illnesses.

Pulmonary alveolar proteinosis (PAP), first described in 1958 (1), is a rare syndrome characterized by the intraalveolar accumulation of surfactant lipids and proteins impairing gas exchange and resulting in progressive respiratory insufficiency. PAP, which has been reported in the medical literature under various terms (alveolar proteinosis, alveolar lipoproteinosis, alveolar phospholipidosis, pulmonary alveolar lipoproteinosis, and pulmonary alveolar phospholipoproteinosis), is recognized to occur in three distinct clinical forms: primary, secondary, and congenital (2, 3). Primary (idiopathic) PAP is a disorder of unknown etiology thought to represent approximately 90% of PAP cases. Secondary PAP occurs as a consequence of any one of a heterogeneous group of underlying clinical conditions (hematologic malignancies; inhalation of toxic dust, fumes, or gases; infectious or pharmacologic immunosuppression; or lysinuric protein intolerance) that impairs alveolar macrophage function, resulting in surfactant accumulation (4). Congenital PAP is a heterogeneous collection of disorders caused by homozygous mutation of the genes encoding surfactant protein (SP)-B, SP-C, and the ABCA3 transporter or by the absence of granulocyte/macrophage colony-stimulating factor (GM-CSF) receptor (5).

Our understanding of PAP pathogenesis has advanced rapidly over the past decade due to a series of contributions from basic, clinical, and translational research. The first real pathogenic clue was provided by the discovery that mice deficient in GM-CSF develop a lung phenotype biochemically, histologically, physiologically, and ultrastructurally indistinguishable from primary PAP (6, 7). Surfactant accumulation in these mice is not due to

increased production but rather to impaired catabolism of surfactant lipids and proteins by alveolar macrophages (8). This and numerous other alveolar macrophage defects were shown to be due to impaired terminal differentiation in mice caused by reduced levels of the GM-CSF-dependent transcription factor, PU.1 (9). GM-CSF also regulates PU.1 levels in human alveolar macrophages (10), which in patients with PAP have defects similar to those of GM-CSF-deficient mice (11). The observation of PAP in GM-CSF-deficient mice was quickly followed by the evaluation of GM-CSF therapy, first in single patient (12) and then in several small series (13–15). GM-CSF deficiency has not been reported in humans (3, 16). However, a second vital pathogenic clue was the observation that primary PAP is specifically and strongly associated with very high levels of GM-CSF autoantibodies (17) that eliminate GM-CSF bioactivity *in vivo* (18). Several methods for measuring GM-CSF autoantibodies have been developed, and an ELISA (19) has demonstrated excellent results (20).

Notwithstanding recent advances, current knowledge about PAP is based on small series and individual case reports. Although data from these studies have been synthesized into a comprehensive review (2), these data represent cases spanning nearly half a century. The incidence, prevalence, or the natural history of primary PAP have not been assessed in a large contemporaneous population. Furthermore, although diagnostic methods have evolved significantly over this period, the strong association of GM-CSF autoantibodies with primary PAP provides a potentially powerful new approach. Novel biomarkers of PAP lung disease also provide additional and potentially useful outcome measures for use in clinical trials to evaluate new therapies.

In July 1999, a national PAP registry was established to more accurately characterize the demographic, clinical, physiologic, radiologic, and serologic features of individuals with PAP in Japan. The present study was not designed to answer questions related to the natural history or responses to therapy; nor was it intended to report on secondary or congenital PAP. Rather, it is intended to provide a cross-sectional evaluation outlining the epidemiology and baseline characteristics of a large contemporaneous group of individuals with primary PAP in Japan at the time of enrollment into the registry. Some of the results of these studies have been previously reported in the form of an abstract (21) and in the proceedings of an international scientific meeting on PAP (22).

METHODS

Study Design

This study was conducted by the Japanese PAP Research Network, which includes nine primary clinical research centers and secondary clinical sites comprising a referral base encompassing the entirety of Japan, a steering committee comprised of the principal investigators at each of the nine primary clinical research centers, and a data collection and analysis center (National Hospital Organization Kinki-Chuo Chest Medical Center, Osaka, Japan). Primary clinical centers included Aichi Medical University, Chiba University, Hokkaido University, Kitazato University, National Kinki-Chuo Chest Medical Center, National Hospital Organization Sanyo Hospital, Nagasaki University, Niigata University Medical and Dental Hospital, and Tohoku University; secondary sites are listed in the APPENDIX. Some investigators in this research network (Y.I., K.N.) are also members of the Rare Lung Diseases Consortium supported by the United States National Institutes of Health. This trial was conducted in three phases: (1) study design and establishment of the Japanese PAP Research Network, (2) participant recruitment and data collection, and (3) data analysis. Three principle components of project were undertaken, including a cross-sectional study (reported here), a natural history study (ongoing), and an evaluation of GM-CSF inhalation therapy (to be reported elsewhere). An independent statistical evaluation of all data was done by the Data and Technology Coordinating Center of

the Rare Diseases Clinical Research Network in the United States. The registry protocol was approved by the institutional review boards of each participating institution, and all participants gave informed consent. At enrollment, the treating physician for each participant completed a case report form and obtained a serum sample, both of which were sent to the data coordinating center for analysis.

Study Participants

Recruitment. Between July 1999 and July 2006, individuals diagnosed with PAP were recruited through the existing physician referral networks at primary or secondary clinical centers and pulmonary clinics and through letters to Japanese pulmonary physicians. Registration into the study was defined as the time of serum collection and completion of the case report form by the referring physician. All patient-related historical, physical examination, and other data were collected within 2 months of registration. To ensure the inclusion of individuals diagnosed with PAP independent of disease severity, patients with PAP were enrolled regardless of whether they were symptomatic. Asymptomatic individuals initially identified by a compatible chest radiograph obtained via mandatory annual health screening programs (students, employees of the government, and registered corporations) in whom a diagnosis of PAP was subsequently confirmed were included. As controls for the measurement of serum GM-CSF autoantibody levels, 24 individuals with other lung diseases (including idiopathic interstitial pneumonias [$n = 10$], sarcoidosis [$n = 9$], and collagen vascular disease [$n = 5$]) and 13 healthy control subjects were recruited into the study from the Kinki-Chuo Chest Medical Center in Osaka.

To evaluate recruitment efficiency, a secondary “intensive screening” was performed in the Niigata prefecture that took advantage of the close relationship among regional pulmonary physicians, 98% of whom trained at and remained affiliated with Niigata University and received weekly departmental communications. All physicians received a recruitment letter and follow-up phone calls as necessary to ensure participation.

Inclusion. All patients with a proven diagnosis of PAP were offered the opportunity to participate in the study regardless of whether they had been previously diagnosed and followed, were currently being followed, or were newly referred. Thus, prevalent and incident cases were enrolled. An acceptable PAP diagnosis was based on histopathologic findings of specimens obtained by open lung biopsy or transbronchial biopsy or on cytologic findings in BAL samples.

Exclusion. Individuals were excluded if they did not have a tissue-proven diagnosis of PAP or if serum was unavailable for analysis of GM-CSF autoantibody levels.

Composition of the study population. Two hundred forty-eight individuals with PAP were enrolled in the study; 11 cases of secondary PAP diagnosed at autopsy were identified during the study period but were excluded from the analysis because serum was unavailable. Of the participants enrolled, 223 had an elevated serum level of GM-CSF autoantibody and no underlying clinical condition known to cause PAP. Twenty-five individuals did not have an elevated serum level of GM-CSF autoantibody (*see below*) but had a condition known to cause PAP secondarily. One individual had neither elevated GM-CSF autoantibody levels nor an underlying explanation for the presence of PAP. These three groups of patients were categorized as autoimmune, secondary, and unclassified PAP, respectively. This report includes the data and analysis for individuals with autoimmune PAP. Data and results for secondary PAP will be reported elsewhere.

Diagnostic Criteria

The diagnosis of PAP was established by the presence of characteristic findings from high-resolution computed tomography (HRCT) of the chest (23) and pathologic and/or cytologic specimens obtained by video-assisted thoracoscopic lung biopsy ($n = 16$), transbronchial lung biopsy ($n = 86$), or bronchoalveolar lavage fluid (BALF)/cell cytology ($n = 218$) (24). The diagnosis of PAP was based on HRCT and bronchoalveolar lavage (BAL) cytology in 58.7% of patients; HRCT, BALF, and transbronchial lung biopsy (TBLB) in 34.1% of patients; and BAL cytology or TBLB and video-assisted thoracoscopic surgery (VATS) in 7.2% of patients. The radiologic features characteristic of PAP on HRCT used here included the presence of a diffuse, patchy, “geographic” pattern of ground glass opacification superimposed on interlobular septal thickening.

ing in multiple lobes (25). The diagnostic features of PAP in pathologic specimens included intraalveolar eosinophilic, periodic acid-Schiff-positive material and in cytology specimens included turbid, periodic acid-Schiff-positive, eosinophilic BALF, and intracellular surfactant inclusion bodies in alveolar macrophages (26, 27). Patients with PAP were enrolled irrespective of whether they were symptomatic at the time of registration.

Epidemiology

Because determining the incidence and prevalence of rare diseases is difficult, strict criteria were used. Only patients enrolled in full years of the study were included in calculations to avoid potential seasonal ascertainment bias. Incidence was determined for each full year of the study (2000–2006) and calculated by dividing the number of individuals newly diagnosed with PAP in a given year by the size of the Japanese population (129,180,548) taken from the governmental census report (WHLW Statistical Database, 2006, Ministry of Health, Labor and Welfare). Prevalence was calculated by dividing the total number of patients with PAP registered during all full years of the study period by the size of the Japanese population.

Pulmonary Function Methodology

Pulmonary function testing was performed by certified pulmonary function technicians. The data collected included FEV₁/FVC, FVC, VC, single-breath diffusing capacity of carbon monoxide (DL_{CO}), and arterial blood gases. Acceptability and reproducibility criteria from the American Thoracic Society recommendations for standardization were used to judge the validity of each testing session (28). Pulmonary function data are presented as the percentage of predicted values. Arterial blood measurements were performed on samples obtained with the patient breathing room air at rest in the supine position.

Disease Severity Score

Participants were assigned a PAP disease severity score (DSS) based on the presence of symptoms and degree of reduction in PaO₂ (both determined at registration) determined with the individual breathing room air in the supine position as previously described (22). The categories included DSS 1 = no symptoms and PaO₂ ≥ 70 mm Hg; DSS 2 = symptomatic and PaO₂ ≥ 70 mm Hg; DSS 3 = 60 mm Hg ≤ PaO₂ < 70 mm Hg; DSS 4 = 50 mm Hg ≤ PaO₂ < 60 mm Hg; DSS 5 = PaO₂ < 50 mm Hg. Qualifying symptoms include dyspnea or cough related to PAP. PaO₂ data were available for 215 of 223 registrants with autoimmune PAP. In the eight registrants in whom PaO₂ was unavailable, oxygen saturation was used to estimate the PaO₂ as follows: oxygen saturation values of 94%, 90%, and 85% were used as the values representing cutoff values of PaO₂ of 70, 60, and 50 mm Hg, respectively.

GM-CSF Autoantibody Measurement

Sera from each participant (patients with PAP or control subjects) were stored frozen (−20°C or −80°C), and GM-CSF autoantibody concentration was measured by ELISA essentially as previously reported (19) with minor modifications as described in the online supplement. Standard antibody was kindly provided by Dr. K. Takada, Hokkaido University.

Serum Biomarker Measurements

Serum biomarkers were evaluated in duplicate samples in blinded fashion in a central laboratory (Y.I.). Serum KL-6, SP-A, and SP-D were measured by ELISA using commercial kits (ED046; Eisai Co. Ltd., Tokyo, Japan; SP-A Test Kokusai-F, International Reagents Co. Kobe, Japan; SP-D ELISA; Yamasa Co., Tokyo, Japan; respectively) as described previously (29–32). Serum carcinoembryonic antigen (CEA) level was measured by radioimmunoassay using monoclonal antibodies (Dainabott, Tokyo, Japan) as described previously (33). Normal serum ranges were KL-6 (<500 U/ml), SP-A (<43.8 ng/ml), SP-D (<110 ng/ml), and CEA (<2.5 ng/ml) (30–34). The normal range for lactate dehydrogenase (LDH) in the clinical laboratory used was <230 IU/L.

Statistics

Numeric data were evaluated for a normal distribution using the Kolmogorov-Smirnov test and for equal variance using the Levene median test. Parametric data are presented as means (±SE), and non-

parametric data are presented as medians and interquartile ranges. Categorical data are presented as a percentage of the total or numerically, as appropriate. Statistical comparisons of parametric numeric data were made with Student's *t* test for two group comparisons if the assumption of equality of variance was satisfied or with the Scatterwaite test if not. Nonparametric numeric data were compared with the Wilcoxon test. Comparisons of categorical data were made with chi-square or Fisher's exact test. The correlation coefficient was obtained using Spearman's correlation method for all data. All tests were two sided, and *P* < 0.05 was considered to indicate statistical significance.

RESULTS

Demographics

Of the 248 patients with histologically and/or cytologically proven PAP enrolled in the Japanese PAP registry, 223 (89.9%) had a positive GM-CSF autoantibody test and were considered to have autoimmune PAP (Figure 1). Patients with a negative GM-CSF autoantibody test had an underlying illness known to cause PAP and were considered to have secondary PAP (9.7%) or were unclassified (0.4%). This article focuses on patients with autoimmune PAP; the features of the latter two groups will be reported elsewhere.

Two-thirds of the patients with autoimmune PAP were men (Table 1). The median age at diagnosis was 51 years, and the mean duration of symptoms at enrollment was 10 months; neither differed according to gender. Patients younger than 10 years of age were rare (Figure 2). Two-thirds of the patients were symptomatic at enrollment, and the proportion did not differ by gender. The age at the time of diagnosis was skewed toward younger individuals due to skewing of the data for men (skewness = 0.0775; *P* = 0.03) but not for women, for whom it was distributed normally (skewness = −0.422; *P* = 0.068) (Figure 2). The bimodal distribution of age at diagnosis for women with PAP, with peaks at ages 25 and 40 years, as previously reported (2), was not observed in this cohort.

Although smoking is a suspected risk factor for PAP, at registration, more than one third of the patients (43%) were never-smokers, a proportion that differed significantly according to gender (83% of women and 24% of men were never-smokers; *P* < 0.001; Table 1). Exposure to dust inhalation, another suspected risk factor for acquired PAP, was present in the

Figure 1. Disposition of the patients with pulmonary alveolar proteinosis (PAP) enrolled into the study. Participants were stratified according to the presence or absence of granulocyte/macrophage colony-stimulating factor (GM-CSF) autoantibodies and then by the presence or absence of an underlying disorder known to cause PAP.

TABLE 1. DEMOGRAPHICS AND DISEASE FEATURES OF PATIENTS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS AT ENROLLMENT ACCORDING TO GENDER

Characteristic	All Patients (n = 223)			Male (n = 151)			Female (n = 72)			P Value
	n	%	Median (IQR)* or Mean $\pm$ SD	n	%	Median (IQR)* or Mean $\pm$ SD	n	%	Median (IQR)* or Mean $\pm$ SD	
Age, yr	223		53 (44–61)	151		52 (44–58)	72		55 (43.5–63)	0.08 [†]
Age at diagnosis, yr	223		51 (41–58)	151		50 (15–89)	72		52 (9–85)	0.064 [†]
Duration of symptoms CXR changes, mo	223		10 (4–36)	151		11 (4–48)	72		9 (4–24)	0.301 [†]
Symptoms	220			147			70			0.754 [‡]
Yes	150	68.4		103	69.6		47	65.3		
No	70	31.8		45	30.4		25	34.7		
Smoking status	217			147			70			<0.001 [‡]
Current smoker	62	28.5		56	37		6	8.5		
Ex-smoker	62	28.5		56	37		6	8.5		
Never-smoker	93	43		35	24		58	83		
Dust exposure	199			137			62			0.001 [‡]
Yes	52	26		44	32		8	13		
No	147	74		93	68		54	87		
Pulmonary function, mean $\pm$ SD										
FVC, % predicted	150		88.0 $\pm$ 18.9	101		90.2 $\pm$ 18.1	49		83.5 $\pm$ 19.9	0.04 [§]
FEV ₁ /FVC	186		84.3 $\pm$ 11.2	128		84.7 $\pm$ 9.5	58		83.5 $\pm$ 14.4	0.57 [§]
VC, % predicted	187		89.4 $\pm$ 19.3	129		90.4 $\pm$ 19.4	58		87.3 $\pm$ 19.1	0.31 [§]
DL _{CO} , % predicted	154		68.6 $\pm$ 26.6	106		70.6 $\pm$ 29.0	48		64.3 $\pm$ 19.5	0.12 [§]
PaCO ₂ , mm Hg	213		39.0 $\pm$ 3.91	145		39.2 $\pm$ 3.8	68		38.6 $\pm$ 4.2	0.31 [§]
PaO ₂ , mm Hg	213		71.8 $\pm$ 13.5	144		71.6 $\pm$ 13.2	69		72.1 $\pm$ 14.1	0.83 [§]
[A–a]DO ₂ , mm Hg	211		31.14 $\pm$ 15.1	144		31.0 $\pm$ 14.9	67		31.3 $\pm$ 15.6	0.90 [§]
GM-CSF autoantibody, μ g/ml	223		15.29 (7.96–26.76)	151		14.91 (7.42–27.03)	72		15.58 (9.34–26.15)	0.51 [†]

Definition of abbreviations: (A–a)DO₂ = alveolar–arterial oxygen gradient; CXR = chest X-ray; DL_{CO} = carbon dioxide diffusing capacity; IQR = interquartile range; PAP = pulmonary alveolar proteinosis.

* Interquartile range is the range from the 25th to the 75th percentiles of the distribution.

[†] Calculated using the Wilcoxon rank sum test.

[‡] Calculated using the chi-square test.

[§] Calculated using Student's *t* test.

histories of only one-quarter (26%) of the patients and differed according to gender (32% of men and 13% of women had a history of exposure; *n* = 199; *P* < 0.001). No instances of autoimmune PAP were familial among any of the 223 individuals, consistent with the absence of a genetic predisposition.

Figure 2. Histogram of the age at diagnosis of autoimmune pulmonary alveolar proteinosis in male (top) and female patients (bottom). Data are grouped into 10-year intervals. The age distribution in female subjects, but not male subjects, was normally distributed (*P* = 0.03 and 0.68, respectively; Kolmogorov-Smirnov test). The median age at diagnosis was similar in male and in female subjects (see Table 1).

Of the pulmonary functions evaluated in patients with autoimmune PAP, the median values for lung volumes (FVC % predicted, VC % predicted) and airflow (FEV₁/FVC) were within the normal range, and only gas transfer (DL_{CO} % predicted) was abnormal (Table 1). Arterial blood gas measurements revealed a similar degree of hypoxemia and elevation of alveolar–arterial oxygen gradient in male and female patients with autoimmune PAP (Table 1).

GM-CSF Autoantibody Levels

GM-CSF autoantibodies levels in the serum were elevated to a similar extent in male and female patients with autoimmune PAP (*P* = 0.51) and were below the level of detection in individuals with secondary PAP; individuals with other lung diseases; disease-free, healthy control subjects (Figure 3); individuals with congenital PAP (*n* = 5, not shown); or individuals with unclassified PAP (*n* = 1, not shown). GM-CSF autoantibody concentrations were skewed toward higher values (skewness = 2.03; *P* < 0.001), a pattern that was similar in men and women (Figure 4). Serum autoantibody concentrations were less than 35 μ g/ml in most patients, a proportion that did not differ by gender (86% for men, 85% for women).

Levels of total serum immunoglobulins were not increased in autoimmune PAP; median (interquartile range [n]) values (in mg/dl) were IgG: 1,323 (1,091–1,468 [83]); IgA: 232 (178–284 [79]); IgM: 101 (71–149 [79]); IgE: 106 (27–219 [60]) mg/dl. In five of these patients, the level of serum IgG exceeded 2,000 mg/dl. Of these, three had intercurrent pulmonary aspergillosis, one had hepatitis C, and one had polymyalgia rheumatica.

Epidemiology

The incidence and prevalence of autoimmune PAP in Japan were evaluated using two approaches, one encompassing nine

Figure 3. Concentration of granulocyte/macrophage colony-stimulating factor (GM-CSF) autoantibodies in the serum at enrollment of individuals with autoimmune pulmonary alveolar proteinosis (PAP) ($n = 223$), secondary PAP ($n = 24$), other lung diseases ($n = 24$), or healthy individuals ($n = 14$). In one individual with unclassified PAP, serum GM-CSF autoantibodies were undetectable (not shown).

nonoverlapping regions representing the entirety of Japan and a second focused to the Niigata prefecture.

In the first approach, incidence was estimated by enumerating individuals registered from all regions of Japan receiving a diagnosis of autoimmune PAP for each full year of the study from 2000 through 2005 (Table 2). Using the Japanese population size in 2005 (129,180,548) taken from the governmental census report (WHLW Statistical Database, 2006, Ministry of Health, Labor and Welfare), the mean ($\pm$ SE) incidence of autoimmune PAP was 0.24 ± 0.03 cases per million population. The total number of patients with autoimmune PAP registered from each of the nine nonoverlapping regions (Hokkaido, Tohoku, Kanto, Hokushinetsu, Tokai, Kinki, Chugoku, Shikoku, and Kyushu) correlated closely with the size of the regional population (Figure 5). The mean ($\pm$ SE) prevalence across all regions was 2.04 ± 0.31 cases per million. These geographic regions range in climate from temperate (Kyushu) to subarctic (Hokkaido), suggesting that climate may not influence the occurrence of autoimmune PAP.

Figure 4. Histogram of serum granulocyte/macrophage colony-stimulating factor (GM-CSF) autoantibody concentrations in male (top) and female (bottom) patients with autoimmune pulmonary alveolar proteinosis. The concentrations were not normally distributed and were significantly skewed toward high values ($P < 0.001$ for both, Kolmogorov-Smirnov test). The distribution of serum GM-CSF autoantibody concentrations was similar in males and female subjects (see Table 1).

TABLE 2. ANNUAL INCIDENCE OF AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS AND ENROLLMENT DURING THE STUDY PERIOD

Year*	Diagnosed		Enrolled [§]
	Japan [†]	Niigata Prefecture [‡]	
1999	10	4	52
2000	30	2	15
2001	14	2	18
2002	26	1	23
2003	43	1	41
2004	43	1	34
2005	31	0	18
2006	22	4	22
Total	219	15	223

* Partial years of enrollment include 1999 (July 1 through December 31) and 2006 (January 1 through June 30).

[†] Includes patients diagnosed in all regions of Japan, the population of which was 129,180,548 as determined in the 2005 Japanese Census report.

[‡] Includes patients diagnosed only in Niigata Prefecture, the population of which was 2,410,000 in 2005.

[§] Enrollment in 1999 includes prevalent cases diagnosed before 1999.

^{||} Includes 11 cases identified in the Japan-wide screen and four additional cases identified only in the intensive screen conducted in the Niigata prefecture.

A second approach used in the Niigata Prefecture took advantage of the especially close relationship and communication among regional pulmonary physicians, 98% of whom received their training at the Niigata University. Intensive screening in this region (population = 2.41 million) resulted in identification of 15 patients with autoimmune PAP during the study period (Table 2). This approach resulted in a mean ($\pm$ SE) incidence of 0.49 ± 0.13 per year (in all full years of the study (2000–2005) and a prevalence of 6.2 cases per million of autoimmune PAP.

Clinical Characteristics

At the time of registration in the study, most patients with autoimmune PAP (69%) were symptomatic, with exertional dyspnea being most common (39%), followed by dyspnea and cough (11%) and cough only (10%) (Table 3). Fever was present in two cases, and weight loss was present in one case. Of the patients with autoimmune PAP, 31% were asymptomatic and were identified by mandatory or voluntary annual health screening programs.

Most patients with autoimmune PAP (65%) had no other intercurrent medical illnesses (Table 4). Among those that did, hypertension was most common (8.5%) and was commensurate with the frequency of hypertension in the Japanese population. Other autoimmune diseases were diagnosed in only three individuals and included polymyalgia rheumatica, hemolytic anemia, and Wegner's granulomatosis. Infections occurred in 12 individuals and included pulmonary aspergillosis in four, atypical mycobacteria in three, mycobacterial tuberculosis in two, pneumonia, hepatitis C, and tinea corporis. The proportion of individuals with intercurrent illness did not vary according to gender. COPD and asthma were underrepresented in our study population (Table 4).

Correlation of Disease Severity with Demographic, Clinical, and Biomarker Data

All patients with autoimmune PAP were stratified by disease severity score (DSS) categories at enrollment as defined in the methods from least severe (DSS-1) to most severe (DSS-5). Roughly one quarter of the patients fell into in each of DSS categories 1, 2, and 3, and one quarter were split between DSS categories 4 and 5 (Table 5).

Pulmonary gas transfer (DL_{CO} %) correlated well with DSS, showing a marked decrease at all successive DSS categories

Figure 5. Distribution and regional prevalence of autoimmune pulmonary alveolar proteinosis (PAP) in Japan at the time of enrollment into the study. (A) Geographic locations of the patients. Shown are the nine separate regions (regional population size from the 2005 Japanese census report) representing all of Japan, including Hokkaido, (5,653,548), Tohoku (11,568,884), Kanto (41,760,805), Hokushinetsu (7,781,254), Tokai (14,894,128), Kinki (20,930,730), Chugoku (7,705,615), Shikoku (4,128,260), and Kyushu (14,757,324). Each solid dot represents one autoimmune PAP patient in the identified region. The Niigata prefecture, in which a second intensive screening for PAP cases was performed, is located within the Hokushinetsu region. The cities of Niigata (triangle), Osaka (circle) and Tokyo (diamond) and the global latitude (in degrees) for each region are indicated for orientation. (B) Correlation of the prevalence of autoimmune PAP cases and regional population size. Hokkaido (solid circle), Tohoku (solid square), Kanto (solid triangle), Hokushinetsu (inverted solid triangle), Tokai (solid diamond), Kinki (solid hexagon), Chugoku (open triangle), Shikoku (open square), Kyushu (open circle).

(Figure 6). Although PAP is often described as a lung disorder with restrictive physiology, the lung volumes (FVC% and VC%) were in the normal range in all patients except those with the most severe disease (DSS-5) (Table 5). Notwithstanding, the DSS correlated weakly with the mild reduction in lung volumes (FVC and VC). The DSS did not correlate with airflow limitation (FEV_1/FVC).

Infections were equally distributed in each of DSS categories 1 through 4, but none occurred in DSS-5. Thus, infections were infrequent in our population and did not correlate with DSS or the degree of impairment in pulmonary function (Table 5).

TABLE 3. SYMPTOMS OF THE PATIENTS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS*

Symptom	Number	%
Asymptomatic	70	31.4
Dyspnea only	87	39.0
Cough only	22	9.9
Sputum only	0	0
Dyspnea + cough	24	10.8
Dyspnea + sputum	3	1.3
Dyspnea + cough + sputum	5	2.2
Other†	9	4.0
Not available	3	1.3

* Complete information was available for 220 individuals.

† Dyspnea and weight loss, or fever, or cough and fever.

The DSS correlated well with selected serum biomarkers, including LDH, SP-A, SP-D, KL-6, and CEA, the strongest of which were the latter two (Table 5). No correlation was observed between DSS and serum GM-CSF autoantibody titer or with antinuclear antibody titer. The DSS was only weakly correlated with age (Table 5) and not with gender, smoking status, history of occupational dust inhalation, or intercurrent medical illness (not shown).

Disease Severity and Progression

Although the present study was cross-sectional in design, data on disease severity and progression since disease onset were collected by the primary treating physician at the time of regis-

TABLE 4. INTERCURRENT MEDICAL ILLNESSES IN INDIVIDUALS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS

Intercurrent Medical Illnesses	Total (n = 212)	Male (n = 143)	Female (n = 69)	P Value
None, n (%)	137 (65)	91 (63.6)	46 (66.7)	0.78
Hypertension, n (%)	18 (8.5)	11 (7.7)	7 (10.1)	0.74
Infections, n (%)*	12 (5.7)	7 (4.9)	5 (7.2)	ND
Hyperlipidemia, n (%)	9 (4.2)	6 (4.2)	3 (4.3)	ND
Diabetes mellitus, n (%)	8 (3.8)	6 (4.2)	2 (2.9)	ND
Inflammatory disorders of the gastrointestinal tract, n (%)††	7 (3.3)	5 (3.5)	2 (2.9)	ND
Liver disease, n (%)‡	7 (3.3)	6 (4.2)	1 (1.5)	ND
Asthma, n (%)	5 (2.4)	1 (0.1)	4 (5.8)	ND
Airflow limitation, n (%)	5 (2.4)	2 (1.4)	3 (2.1)	ND
Allergic rhinitis, n (%)	3 (1.4)	3 (2.1)	0 (0)	ND
Cancer, n (%)¶	4 (1.9)	4 (2.8)	0 (0)	ND
Autoimmune disorders, n (%)**	3 (1.4)	1 (0.1)	2 (2.9)	ND
Interstitial lung disease, n (%)††	3 (1.4)	3 (2.1)	0 (0)	ND
Stroke, n (%)	3 (1.4)	3 (2.1)	0 (0)	ND
Schizophrenia, n (%)	3 (1.4)	2 (1.4)	1 (1.5)	ND
Other disorders‡‡	(<1)	(<1)	(<1)	ND

Definition of abbreviation: ND = not determined.

* Diseases include pulmonary aspergillosis (n = 4), atypical mycobacteria (n = 3), tuberculosis (n = 2), pneumonia (n = 1), hepatitis C (n = 1), and tinea corporis (n = 1).

† Values shown are calculated using the chi-square test. For some diseases, the number of expected values was too low to be evaluated statistically.

‡ Diseases include oral ulceration (n = 1), esophagitis (n = 2), gastritis (n = 1), gastric ulcer (n = 1), duodenal ulcer (n = 1), and ulcerative colitis (n = 1).

§ Diseases include cirrhosis (n = 3), fatty liver (n = 3), and liver dysfunction (n = 1).

|| Defined as individuals with $FEV_1/FVC < 0.7$.

¶ Diseases include cancer of the lung (n = 1), colon (n = 1), prostate (n = 1), and thyroid (n = 1).

** Diseases include polymyalgia rheumatica (n = 1), hemolytic anemia (n = 1), and Wegner's granulomatosis (n = 1).

†† Diseases include pulmonary fibrosis (n = 2) and interstitial pneumonitis (n = 1).

‡‡ Diseases include heart failure (n = 2), Alzheimer's (n = 1), obstructive sleep apnea (n = 1), monoclonal hypergammaglobulinemia (n = 1), hypothyroidism (n = 1), angina (n = 1), membranous nephropathy (n = 1), and Parkinson's disease (1).

TABLE 5. CORRELATION OF DISEASE SEVERITY SCORE WITH THE AGE AT DIAGNOSIS, SYMPTOMS, INFECTIONS, PULMONARY FUNCTION, AND SERUM BIOMARKERS IN PATIENTS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS

Characteristic	Disease Severity Score*					<i>R</i> [†]	<i>P</i> Value [‡]
	1 (<i>n</i> = 58)	2 (<i>n</i> = 60)	3 (<i>n</i> = 55)	4 (<i>n</i> = 37)	5 (<i>n</i> = 12)		
Age at diagnosis, yr	48 (34–55)	46 (36–56)	55 (45–59)	53 (46–59)	58 (53–65)	0.25	<0.001
Symptomatic, %	0	100.00	78.18	94.59	100.00	0.64	<0.001
Infection, <i>n</i>	3	3	3	3	0	0.01	0.887
<i>Aspergillus</i> spp., <i>n</i>	2	0	1	1	0		
<i>Mycobacterium</i> spp., <i>n</i>	1	1	1	2	0		
Other [§]	0	2	1	0	0		
Pulmonary function							
FVC, % predicted	97.1 ± 17.3	92.3 ± 17.2	81.9 ± 21.4	82.0 ± 13.1	76.4 ± 19.0	−0.36	<0.001
FEV ₁ /FVC	82.4 ± 7.9	84.8 ± 10.9	86.0 ± 8.3	85.7 ± 10.7	79.3 ± 27.3	0.16	0.034
VC, % predicted	98.8 ± 16.3	91.3 ± 20.6	87.4 ± 19.2	81.5 ± 14.4	76.1 ± 23.7	−0.35	<0.001
DL _{CO} , % predicted	86.8 ± 23.2	72.4 ± 24.5	64.9 ± 24.1	50.4 ± 18.0	43.1 ± 20.4	−0.56	<0.001
Biomarkers							
GM-CSF autoantibody, µg/ml	14.8 (7.78–25.62)	16.3 (7.49–27.03)	12.75 (6.46–27.38)	16.34 (11.82–25.77)	13.21 (7.70–25.88)	0.004	0.947
LDH, IU/ml	233 (199–365)	316 (247–454)	324 (253–445)	361 (266–474)	406 (247–538)	0.29	<0.001
CEA, ng/ml	1.6 (1.0–2.3)	3.0 (1.8–5.7)	4.7 (2.65–6.85)	5.6 (3.9–12)	17.1 (6–23)	0.53	<0.001
SP-A, ng/ml	48.4 (31.0–74.3)	80.1 (51.3–131)	106 (63.3–143)	133 (78.8–237)	162 (85.4–302)	0.45	<0.001
SP-D, ng/ml	126 (75.9–170)	193 (103–298)	211 (121–324)	268 (183–426)	233 (159–253)	0.37	<0.001
KL-6, U/ml	1,120 (739–2,480)	2,676 (1,585–8,200)	4,030 (2,000–8,330)	7,410 (4,290–12,600)	15,100 (2,150–24,400)	0.52	<0.001
ANA, 1/dilution	0 (0–20)	0 (0–20)	0 (0–40)	0 (0–20)	0 (0–40)	0.03	0.73

Definition of abbreviations: ANA = antinuclear antibody; CEA = carcinoembryonic antigen; GM-CSF = granulocyte/macrophage colony-stimulating factor; LDH = lactate dehydrogenase; SP-A = surfactant protein A; SP-D = surfactant protein D.

* Data are for all participants evaluated at enrollment and are presented as median (interquartile range) or mean ± SE or as percent affected or number of cases where indicated.

[†] Spearman correlation coefficient.

[‡] Values calculated using the Spearman correlation test.

[§] Includes hepatitis C (disease severity score [DSS]-3), unspecified pneumonia (DSS-2), and ringworm (DSS-2).

tration. To determine if the severity of autoimmune PAP at registration correlated with its duration, patients were stratified into three groups in which the time between onset and registration was as follows: (1) ≤1 year, (2) >1 year and ≤10 years, or (3) >10 years. No differences were observed among these three groups with respect to (1) the proportion of symptomatic individuals, (2) the proportion of individuals falling into each DSS category, (3) pulmonary function (FVC % predicted, VC % predicted, FEV₁/FVC, DL_{CO} % predicted) (Table 6), or (4) most serum biomarkers (GM-CSF autoantibody level, CEA, SP-A, KL-6, and antinuclear antibody) (not shown). A weak correlation of LDH and SP-D with the duration of disease in patients with autoimmune PAP was observed (not shown).

In two-thirds of the recently diagnosed individuals (group 1), symptoms were unchanged from the time of onset to registration (Table 6). In those with disease of intermediate duration (group

2), 42.5% had improved, 29.8% had worsened, and 27.7% were unchanged. In those with prolonged disease (group 3), nearly two-thirds had worsened, and one-quarter were unchanged. This result reflects the various and in some cases multiple treatment approaches used in these individuals.

Our study design also permitted enrollment of asymptomatic individuals with autoimmune PAP. Among those asymptomatic at registration, 60% of those recently diagnosed, 74.1% with an intermediate duration of disease, and 33.3% with prolonged disease were unchanged since onset (Table 6). Despite the absence of whole-lung lavage therapy for PAP, 11 of 39 (28.2%) had subclinical disease or had undergone spontaneous improvement and were asymptomatic at the time of registration.

Correlations with Serum GM-CSF Autoantibody Concentration

To determine if the severity of the pulmonary abnormalities was correlated with the level of GM-CSF autoantibody, patients with autoimmune PAP were grouped into quartiles according to their serum GM-CSF autoantibody level (Q1–Q4 = <7.9, 8–15.2, 15.3–26.8, and >26.8 µg/ml, respectively). GM-CSF autoantibody levels in serum did not correlate with duration of disease, DSS, pulmonary function (FVC%, VC%, FEV₁%, DL_{CO}%), or serum biomarkers (LDH, SP-A, SP-D, CEA, KL-6) (see Table E1 in the online supplement). Even in individuals with GM-CSF autoantibody levels above 35 µg/ml (the top 13% of individuals), no correlations were observed in any of these parameters (not shown). The serum GM-CSF autoantibody also did not correlate with age, gender, smoking status, a history of environmental or occupational dust inhalation exposure, or duration of disease (not shown).

Figure 6. Correlation of the disease severity score with carbon monoxide diffusing capacity (DL_{CO}) in patients with autoimmune pulmonary alveolar proteinosis. Results are shown as mean (±SE) for individuals classified as disease severity score (DSS)-1 (*n* = 42), DSS-2 (*n* = 38), DSS-3 (*n* = 27), DSS-4 (*n* = 28), or DSS-5 (*n* = 12).

DISCUSSION

In this study, we report on the epidemiologic, demographic, clinical, pulmonary function, and serum biomarker data from

TABLE 6. CORRELATION OF DURATION OF DISEASE WITH CLINICAL PROGRESSION AND PULMONARY FUNCTIONS IN PATIENTS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS

Characteristic	Duration of Disease*			P Value†
	≤1 yr (n = 124)	>1 yr, ≤10 yr (n = 89)	>10 yr (n = 10)	
Disease severity score				0.11
1, % of patients	29.84	20.22	30.00	
2, % of patients	28.23	25.84	20.00	
3, % of patients	22.58	26.97	40.00	
4, % of patients	14.52	20.22	10.00	
5, % of patients	4.84	6.74	0.00	
Pulmonary function				
FVC, % predicted	89.7 ± 18.4	86.6 ± 18.9	80.0 ± 26.2	0.10
FEV ₁ /FVC	84.5 ± 12.0	84.6 ± 10.6	80.4 ± 8.4	0.12
VC, % predicted	91.0 ± 19.0	87.6 ± 19.4	86.5 ± 23.5	0.08
D _{LCO} , % predicted	71.5 ± 27.7	64.3 ± 24.9	72.1 ± 26.0	0.26
Symptomatic, %	65.0	72.4	70.0	0.28
Disease progression‡				
Symptomatic individuals	(n = 50)	(n = 47)	(n = 8)	0.002
Improved, n	11	20	1	
Worsened, n	6	14	5	
No change, n	33	13	2	
Asymptomatic individuals§	(n = 15)	(n = 21)	(n = 3)	0.99
Improved, n	5	4	2	
Worsened, n	1	2	0	
No change, n	9	15	1	

Definition of abbreviation: D_{LCO} = carbon dioxide diffusing capacity.

* Data are for all participants at enrollment and are presented as percent, median (interquartile range), or mean ± SE.

† Values shown are calculated using the Spearman correlation test.

‡ Treating physician's clinical impression of the severity of pulmonary disease progression from the time of onset of symptoms or an abnormal chest radiograph to the time of enrollment in the study.

§ None of the individuals who were asymptomatic at registration had previously received whole-lung lavage or granulocyte/macrophage colony-stimulating factor aerosol therapy.

the largest contemporaneous cohort of patients with autoimmune PAP assembled to date.

Several lines of evidence support the use of the term “autoimmune PAP” and the stratification of PAP into autoimmune and secondary forms. First, underscoring the importance of GM-CSF in pulmonary surfactant homeostasis, mice deficient in GM-CSF (6, 7) or its receptor (35) develop a pulmonary phenotype biochemically, histologically, physiologically, and ultrastructurally identical to autoimmune PAP in humans (36). Second, GM-CSF autoantibodies seem to be critical to the pathogenesis of autoimmune PAP because high levels are strongly associated with it but are not present in secondary or congenital PAP, other lung diseases, or in healthy individuals (17). Their binding affinity for GM-CSF (~20 pmol/L) is higher than the GM-CSF receptor in its low- (~3,200 pmol/L) or high-affinity (120 pmol/L) binding state (37), and they eliminate GM-CSF bioactivity *in vivo* (18). Third, transfer of purified GM-CSF autoantibodies from patients with PAP into blood from healthy individuals reproduces the myeloid cell abnormalities observed in patients with autoimmune PAP (38). Fourth, anti-murine GM-CSF antibodies reproduce these abnormalities in wild-type mice. Fifth, the courses of autoimmune and secondary PAP are different: Secondary PAP has a far worse outcome (H. Ishii, in preparation).

Important findings of this study are the incidence and prevalence data for autoimmune PAP in Japan. Using an intensive screening approach involving 98% of pulmonary physicians in one region, the highest incidence and prevalence estimates were 0.49 and 6.2 cases per million, respectively. Our prevalence value is higher than reported for Israel (3.7 per million) (39) and lower

than for the United States (~10–40 cases per million) (40). One of 15 cases in the former study was congenital PAP, and the latter study included all three clinical forms. If we included all types of PAP identified in Japan during the study period, the prevalence would be 8.7 per million. We did not observe familial clustering in our study, in contrast to the Israeli report in which PAP occurred in two siblings. These likely represent congenital PAP, which occurs secondary to mutations in the genes encoding SP-B (41), surfactant protein C (42), or ABCA3 transporter protein (43). Of the patients with autoimmune PAP in our study, 31% were asymptomatic and were identified by annual medical screening programs. Our observations suggest that the true incidence and prevalence of autoimmune PAP is higher than reported and show that nearly a third of cases are subclinical. Although regional differences in may exist, more studies are needed.

The demographics of Japanese patients with autoimmune PAP differ in several respects from a retrospective meta-analysis done by Seymour and colleagues, which includes most or all cases of PAP reported in the medical literature as of 2002 (2). First, the median age at diagnosis was 51 years and similar in men and women, in contrast to Seymour and colleagues' report in which the median age was 39 years and was different in men and women (39 and 35 years respectively). Second, the age at diagnosis was normally distributed in women and did not have the bimodal pattern previously reported (2). Third, the male:female ratio (2.10:1.0) and, fourth, the proportion of current smokers (28.5%) were lower than previously reported (2.65:1.0 and 72%, respectively) (2). The absence of a male predominance among non-smokers (never- and ex-smokers) in our study (male:female ratio = 0.60:1.0) is similar to the prior report (0.69:1.0) (2) and is consistent with the possibility that the high proportion of men among patients with PAP may be explained by their higher frequency of tobacco use. However, a high proportion of women in our cohort had no history of smoking (83%) or occupational exposure (87%), suggesting that another factor may be involved in the etiology of autoimmune PAP. Our study did not address potential effects of passive smoke exposure.

It is surprising that COPD was not recognized more commonly in our cohort given that the proportion of current, ex-, and never-smokers was similar to the Japanese population in whom COPD occurs in 8.6% (44, 45). Using the same criterion (FEV₁/FVC < 0.70) as Fukuchi and colleagues (44), only five individuals (2.7%) in our cohort had airflow limitation, whereas 24 individuals were expected of having airflow limitation. Of these, two were male (one ex-smoker, aged 55 yr; one never-smoker, aged 71 yr), and three were female (one current smoker, aged 39 yr; one ex-smoker, aged 28 yr; and one never-smoker, aged 44 yr). Although the reason for this is not clear, it is interesting that asthma, another common lung disorder with an inflammatory component of pathogenesis, was also underrepresented in our cohort (observed frequency = 2.4%; expected frequency ~8.2%). PAP may alter the phenotype of disorders with an inflammatory component of the pathogenesis. This is supported by observations that GM-CSF is required for myeloid cell functions in humans (38) and mice (9), where it regulates a number of innate immune responses, including the TLR4 response to lipopolysaccharide (46), and GM-CSF autoantibodies in patients with PAP virtually eliminate GM-CSF bioactivity (18). Thus, GM-CSF autoantibodies may blunt inflammatory responses in patients with PAP, which may affect tissue destruction in COPD and the tendency for exacerbations in asthma.

The DSS (22) provided a useful measure of lung disease severity in symptomatic and asymptomatic autoimmune patients with PAP, which was important because nearly one-third of the patients were asymptomatic and because dyspnea is difficult to quantify in PAP due to the insidious onset. A limitation of this

study was the absence of a dyspnea index. Notwithstanding, the DSS correlated well with the DL_{CO} % predicted, less well with FVC % predicted and VC % predicted, and not with FEV₁/FVC. Although PAP is usually described as a restrictive lung disease, reductions in lung volumes in autoimmune PAP were minor and fell in the normal range in most patients, suggesting that these pulmonary function measures may be of limited usefulness in assessing the severity of PAP lung disease. Physiologically insignificant restriction is further supported by the absence of hypoventilation, even in severe cases. Thus, in autoimmune PAP, hypoxemia is primarily due to reduced oxygen diffusion and possibly ventilation-perfusion mismatching.

Infections were less common among Japanese PAP registrants than previously reported (2). Furthermore, although *Nocardia* was identified in 60% of reported PAP cases complicated by infection (2), no cases of *Nocardia* infection were observed in our study during the period of observation. It is possible that these differences represent reporting bias or differences in clinical care of early infections because a number of the prior reports reflect infectious complications occurring over four decades.

Our observation that GM-CSF autoantibody levels did not correlate with disease severity as measured by the presence of symptoms, pulmonary function testing, or the DSS is consistent with prior reports (2, 3, 22). Because GM-CSF autoantibodies in patients with PAP are polyclonal, it is possible that measuring the level of neutralizing antibody may provide a better correlation with disease severity. We have reported a patient with autoimmune PAP in whom serial measurement of serum GM-CSF neutralizing activity correlated well with disease severity (47). Furthermore, the serum GM-CSF neutralizing activity was reduced in a patient who was successfully treated with inhaled GM-CSF (47). Pulmonary compartmentalization of GM-CSF antibodies may be important in determining disease severity and could explain the lack of correlation with serum autoantibody levels. Neither the autoantibody levels, proportion of symptomatic individuals, pulmonary functions, nor DSS correlated with the duration of disease, which is consistent with the concept that disease severity does not worsen with time in most patients.

The method used to measure GM-CSF autoantibody levels is similar to prior reports (17) except that a new monoclonal GM-CSF autoantibody standard was used. Although this standard yields reproducible results, it yields autoantibody levels one seventh that of the previous GM-CSF autoantibody standard isolated from pooled PAP serum (3, 15, 17, 19) and similar in sensitivity and specificity. Our results support the use of GM-CSF autoantibody measurement in the diagnosis of autoimmune PAP as an adjunct to chest CT and bronchoscopy.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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APPENDIX

The patients were referred to one of the nine primary clinical research centers by the following physicians (only one individual is listed per institution because of space limitations): Arata Azuma (Nippon Medical School), Masato Katagiri (Kitazato University), Masafumi Nijima (Narita Red Cross Hospital), Shinobu Akagawa (NHO Tokyo Hospital), Masayuki Nara (Tohoku University), Akira Fujita (Metropolitan Fuchu Hospital), Ryo Takahashi (Osaka Prefectural Medical Center for Respiratory and Allergic Diseases), Jun Sato (Hamamatsu University School of Medicine), Hidenori Ichiyasu (Kumamoto University), Yoshihiro Honda (Sendai Kohsei Hospital), Yoshio Taguchi (Tenri Hospital), Masakazu Aitani (NTT West Osaka Hospital), Masanori Nakanishi (NHO Tsuruga Hospital), Tetsuo Yamaguchi (JR Tokyo General Hospital), Muneharu Maruyama (Toyama University Hospital), Atsuhiko Fujii (Juntendo University), Kohei Yamauchi (Iwate Medical University), Towako Nagata (Nagasaki University), Tatsuro Mikawa (Yodogawa Christian Hospital), Toshihiko Hashizume (Yokohama Kyosai Hospital), Sakae Honnma (Toranomon Hospital), Masato Tohyama (University of the Ryukyus), Masaharu Nagayama (Shizuoka City Hospital), Noriharu Shijubo (Sapporo Medical University), Koichiro Takahashi (Saga Medical University), Iwao Komuro (Metropolitan Hiroo Hospital), Mihoko Doi (Hiroshima University), Kaoru Maki (Matsue Seikyo Hospital), Yoshitsune Sando (Gunnma University), Hiroo Miyazaki (Fukuroi Municipal Hospital), Youkou Shibata (Yamagata University), Hirohisa Toga (Kanazawa Medical College), Naotoshi Suruta (NHO Wakayama Hospital), Hiroaki Kume (Nagoya University), Ken Nawa (Hitachi General Hospital), Kaneo Kawazoe (Naka Tsushima Hospital), Watako Takehara (Chubu Rosai Hospital), Yasuhiro Ieda (Kinki University Sakai Hospital), Masaru Yauchi (Ishimaki Red Cross Hospital), Yuji Akiba (Asahikawa Kosei Hospital), Masako Toh (The Fraternity Memorial Hospital), Toshiyuki Yamauchi (Keihai Rosai Hospital), Yuzuru Inoue (Shin Yamate Hospital), Kenji Kohno (Kyoto Prefectural University of Medicine), Machiko Arita (Kurashiki Central Hospital), Kazunari Himeno (Fujita Health University), Nobuto Kishimoto (Takamatsu Municipal Hospital), Masaya Yamasato (NHO Minami Yokohama Hospital), Aya Sugawara (Fukushima Medical University), Atuko Kobayashi (Saiseikai Suita Hospital), Katsunori Sugisaki (Oita University), Kenichiro Ohtani (Osaka City University Medical School), Yoshikazu Ishii (Jichi Medical University), Yoshiki Kobayashi (Takatsuki Red Cross Hospital), Shigeru Koyama (Nagano Red Cross Hospital), Hiroko Kimura (Tohoku Rosai Hospital), Atuhiko Goto (Okazaki City Hospital), Amihiko Hirano (Wakayama Medical University), Jun Shiraki (Kochi Health Science Center), Fumiko Sugatani (Teine Kijinkai Hospital), Akira Miyashita (Yokohama City University School of Medicine), Momoyo Ukai (Tokushima University), Yoshida Makino (Osaka Medical College), Hidenori Mori (Gifu University), Susumu Oguri (NHO Minami Kyoto Hospital), Taku Inoue (Sano Kousei Sougou Hospital), Masaaki Takahashi (Asahikawa Medical University), Michihiro Yoshimi (Kyusyu University), Toshiaki Hidaka (Koga Sogo Hospital), Masahiko Iwaoka (Fujieda Municipal Central Hospital), Daizen Cho (Tsubame Rosai Hospital), Eishi Ito (Hakodate City Hospital), Hiroyo Okurakata (Saiseikai Sanjo Hospital), Hiroshi Saiki (NHO Miyazaki Hospital), Jun Katsuta (Takayama Red Cross Hospital), Yoshifumi Imazu (Miyazaki University), Mikiko Ono (Kagoshima University), Tatsuya Hosono (Jichi Medical College), Shinji Takeuchi (Takamatsu Red Cross Hospital), Kenji Konishi (Seirei Hamamatsu General Hospital), Mikio Oka (Kawasaki Medical University), Takefumi Saito

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