CHEST

Original Research

DIFFUSE LUNG DISEASE

Duration of Benefit in Patients With Autoimmune Pulmonary Alveolar Proteinosis After Inhaled Granulocyte-Macrophage Colony-Stimulating Factor Therapy

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Background: **Treatment of autoimmune pulmonary alveolar proteinosis (aPAP) by subcutaneous injection or inhaled therapy of granulocyte-macrophage colony-stimulating factor (GM-CSF) has been demonstrated to be safe and efficacious in several reports. However, some reports of subcutaneous injection described transient benefit in most instances. The durability of response to inhaled GM-CSF therapy is not well characterized.**

Methods: **To elucidate the risk factors for recurrence of aPAP after GM-CSF inhalation, 35 patients were followed up, monitoring for the use of any additional PAP therapies and disease severity score every 6 months. Physiologic, serologic, and radiologic features of the patients were analyzed for the findings of 30-month observation after the end of inhalation therapy.**

Results: **During the observation, 23 patients remained free from additional treatments, and twelve patients required additional treatments. There were no significant differences in age, sex, symptoms, oxygenation indexes, or anti-GM-CSF antibody levels at the beginning of treatment between the two groups. Baseline vital capacity (% predicted, %VC) were higher among those who required additional treatment ($P < .01$). Those patients not requiring additional treatment maintained the improved disease severity score initially achieved. A significant difference in the time to additional treatment between the high %VC group (%VC ≥ 80.5) and the low %VC group was seen by a Kaplan-Meier analysis and a log-rank test ($P < .0005$).**

Conclusions: **These results demonstrate that inhaled GM-CSF therapy sustained remission of aPAP in more than one-half of cases, and baseline %VC might be a prognostic factor for disease recurrence.**

Trial registry: **ISRCTN Register and JMACCT Clinical Trial Registry; No.: ISRCTN18931678 and JMAIIA00013; URL: http://www.isrctn.org and http://www.jmacct.med.or.jp**

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Abbreviations: A-aDO_2 = alveolar-arterial oxygen difference; Ab = antibody; aPAP = autoimmune pulmonary alveolar proteinosis; AT = additional treatment; BALF = BAL fluid; CEA = carcinoembryonic antigen; DLCO = diffusing capacity of the lung for carbon monoxide; DSS = disease severity score; FR = free from additional treatment; GM-CSF = granulocyte-macrophage colony-stimulating factor; IQR = interquartile range; KL-6 = Krebs von den Lungen-6; LDH = lactate dehydrogenase; PAP = pulmonary alveolar proteinosis; ROC = receiver operating characteristics curve; SP = surfactant protein; VC = vital capacity; WLL = whole-lung lavage

Autoimmune pulmonary alveolar proteinosis (aPAP) is a rare lung disease characterized by the accumulation of surfactant protein (SP), which causes progressive respiratory insufficiency.[1-3] The pathogenesis has been attributed to the excessive production of a neutralizing autoantibody against granulocyte-macrophage colony-stimulating factor (GM-CSF) that impairs GM-CSF-dependent surfactant clearance mediated by

alveolar macrophages.[4-8] On pulmonary function testing, the most common pattern seen is that of a restrictive defect, with a disproportionate reduction in diffusing capacity of the lung for carbon monoxide (DLCO) relative to a modest impairment of vital capacity (VC).[2] The disease is usually treated by whole-lung lavage (WLL), which remains the standard therapy to date.

The first patient successfully treated with subcutaneously administered GM-CSF was reported in 1996.[9] In an international multicenter phase 2 trial study, 14 patients were treated with GM-CSF by subcutaneous injection in escalating doses over a 3-month period, with an overall response rate of 43%.[10,11] A subsequent single-center study of 21 patients with aPAP treated with GM-CSF by subcutaneous administration in escalating doses for 6 to 12 months reported an overall response rate of 48%.[12] Several single cases of subcutaneous GM-CSF therapy have reported similar outcomes.[13,14] However, local reaction at sites of injection and other minor toxicities occurred in 85% of patients receiving subcutaneous GM-CSF.[2]

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GM-CSF inhalation is a promising alternative therapy for aPAP that has been demonstrated to lead to functional, biologic, and radiologic improvement.[15-18] Our national, multicenter phase 2 study revealed that the therapy reduced alveolar-arterial oxygen difference (A-aDO_2) by 12.3 mm Hg in 35 patients who completed the therapy, resulting in 24 responders. No treatment-related side effects were noted. Of importance, our previous phase 2 study showed that there was no significant difference in serologic, physiologic, and CT scan testing, except for serum Krebs von den Lungen-6 (KL-6) levels, between the responders and the nonresponders.[18]

There is limited information regarding the duration of benefit after various treatments of aPAP. In the literature analysis of 55 cases with a therapeutic response to WLL, the median duration of clinical benefit from lavage was 15 months.[2] A phase 2 study of subcutaneous GM-CSF administration demonstrated that 45% of patients required WLL during follow-up observation of 39 ± 17.3 months.[12] In a retrospective analysis of inhaled GM-CSF therapy (250 μg bid), five of 12 patients manifest progressive disease during observation.[17] As the disease progresses very slowly and can fluctuate in some cases, it is necessary to evaluate the prognosis by monitoring prospectively at the same time points after the treatment and by disease severity score as well as the need for additional treatment. The aim of this study was to define the duration of benefit among patients who underwent GM-CSF inhalation therapy.

Materials and Methods

Patients and Protocols

The present study prospectively observed patients who participated in a multicenter phase 2 trial (35 patients, registered as ISRCTN18931678 and JMAIIA00013) of GM-CSF inhalation therapy described previously. In brief, patients who had lung biopsy or cytologic findings diagnostic for pulmonary alveolar proteinosis (PAP), including elevated serum anti-GM-CSF antibody (Ab) levels and no improvement during a 12-week observation period, entered the treatment phase. Recombinant human GM-CSF dissolved in 2 mL of sterile saline was inhaled using an LC-PLUS nebulizer (PARI International). The treatment consisted of high-dose GM-CSF administration (125 μg bid on days 1-8, none on days 9-14; sargramostim) for six repetitions of 2-week cycles, then low-dose administration (125 μg once daily on days 1-4, none on days 5-14) for six repetitions of 2-week cycles (for a total dose of 15 mg). The clinical information including physiologic, serologic, and radiologic features obtained[18] was compared with the results of the following 30-month observation.

Patients were regularly evaluated by their physicians at the network hospitals after the GM-CSF inhalation therapy. The worsening dyspnea was evaluated with pulse oximetry, arterial blood gas analysis, or both in outpatient settings. Disease severity in patients was evaluated using PAP disease severity score (DSS) described previously.[19] Patients underwent additional treatments based on

either of the following criteria: (1) DSS is 3 or 4 and symptoms are worsening or (2) DSS 5, as shown in Figure 1. The consortium office of Niigata University contacted the network hospitals every 6 months with a questionnaire regarding additional treatment and disease severity score of the patient. The follow-up clinical information obtained at each network hospital was entered into a database to be compared with the results of the baseline clinical evaluation of each patient. The data were collected from nine clinical research centers in Japan (Hokkaido University, Tohoku University, Chiba University, Kitasato University, Niigata University, Aichi Medical University, National Hospital Organization Kinki-Chuo Chest Medical Center, National Hospital Organization Yamaguchi-Ube Medical Center, and Nagasaki University Institute of Tropical Medicine).

The study was approved by institutional review board of Niigata University (approval No. NH17-006) and the institutional review boards at all participating centers. Informed consent was obtained from all control subjects. The clinical information obtained by the clinical studies was entered into a database to be compared with the results of the 30-month observation. The study was designed and monitored for data quality and safety by a steering committee composed of the principal investigator at each participating site. The steering committee held a conference twice a year, where the findings of the observation were monitored.

BAL Procedures and GM-CSF Autoantibodies

The steering committee edited a standard operational procedure for BAL, which was followed by all participating institutes and described previously.[18,20] The concentration of GM-CSF autoantibodies in BAL fluid (BALF) or in serum were measured using a sandwich enzyme-linked immunosorbent assay as described previously.[4,21]

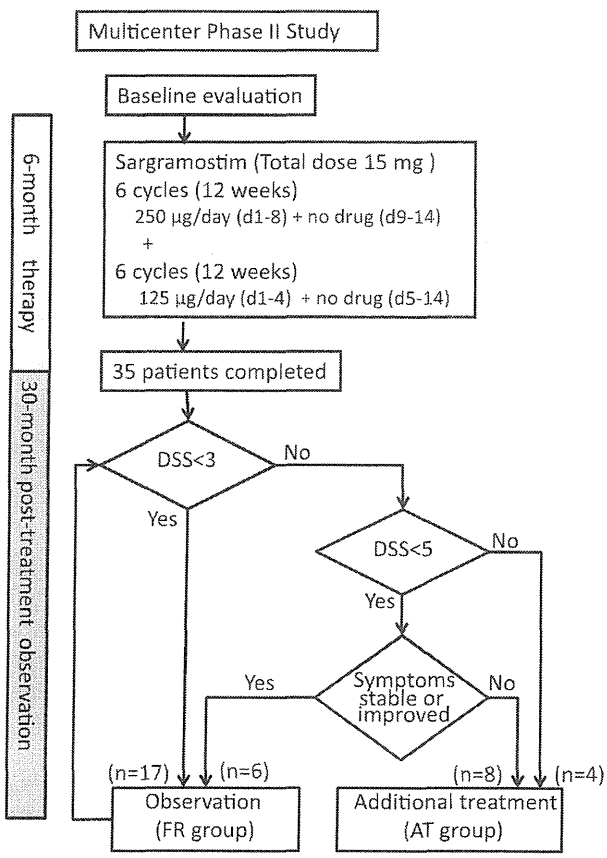

FIGURE 1. Profile of the study cohort. AT = additional treatment; DSS = disease severity score; FR = free from additional treatment.

Statistical Analysis

Numerical results are presented as the mean ± SE or the median and interquartile range (IQR). The χ^2 test was used to evaluate proportions for variables between high and low responders. The paired *t* test was used for comparisons between normally distributed data and the treatment periods. Comparisons of nonparametric data were made using the Wilcoxon signed-rank test. For group comparisons, unpaired *t* tests and Wilcoxon rank-sum tests were used. All *P* values were reported as two-sided. Analysis was performed using JMP software, version 8.0.2 (SAS Institute Inc).

RESULTS

Patient Characteristics and Requirements for Additional Treatments as an Indicator of Recurrence

Demographic data of patients are shown in Table 1. During the 30 months of observation after the end of GM-CSF inhalation, the need for treatments was monitored as an indicator of disease recurrence in each patient. Twenty-three patients were free from additional treatments during 30 months of observation and were designated as FR (free from additional treatment). Twelve patients who required additional treatments, including six patients with recurrence described in our previous study,[18] were designated as AT (additional treatment). Of those, two patients maintained most severe disease (DSS 5) even after the GM-CSF treatment and underwent subsequent WLL. One patient who had dyspnea, cough, and sputum production did not respond to the GM-CSF treatment and underwent subsequent WLL. One patient with cough and dyspnea showed worsening in PaO_2 and cough and had WLL 12 months after the GM-CSF inhalation. The other eight patients with dyspnea showed worsening in PaO_2/oxygen saturation by pulse oximetry (two patients worsened to DSS 5) and underwent additional therapy (e-Fig 1); five underwent additional GM-CSF inhalation treatments, two had WLL, and one patient, a nonresponder, declined WLL and underwent acetylcysteine inhalation, showing much improvement in hypoxia. Median time to additional treatment of the 12 patients was 50.5 weeks, with a range of 8.5 to 117.5 weeks. There was no significant difference in age, sex, symptoms, smoking status, history of dust exposure, arterial blood gas analysis, numbers of responders to GM-CSF inhalation, history of previous lung lavage, and anti-GM-CSF-Ab titer between the FR and AT groups (Table 1). There was no significant difference in disease markers, including baseline levels of PaO_2, $A\text{-}aDO_2$, %VC, %DLCO, CT scan scores, lactate dehydrogenase (LDH), and KL-6 between the patients who underwent WLL (n = 6, AT-WLL group) and those treated with GM-CSF inhalation (n = 5, AT-GM group)

Table 1—*Baseline Clinical Characteristics of Patients Free From Additional Treatment and Those Who Required Additional Treatment After GM-CSF Inhalation*

Characteristic	FR (n = 23)			AT (n = 12)			*P* Value
	No.	%	Median (IQR) or Mean ± SE	No.	%	Median (IQR) or Mean ± SE	
Age, y	23	...	52.5 (48-61)	12	...	52.5 (41.75-58)	.33[a]
Sex			...			...	.54[b]
Female	9	39		6	50		
Male	14	61		6	50		
Responders	17	74	...	7	58	...	.35[b]
Duration of symptoms, mo	23	...	20 (11-61)	12	...	18 (7.75-72)	.78[a]
Symptoms			...			...	
Dyspnea	22	96		12	100		.36[b]
Cough	10	43		7	58		.65[b]
Sputum	8	35		4	33		.71[b]
Smoking status			...			...	.39[b]
Current smoker	8	35		2	17		
Ex-smoker	5	22		2	17		
Never smoked	10	43		8	67		
Dust exposure	22	...	...	11	...	...	.27[b]
Yes	8	36		3	18		
No	14	64		8	82		
Arterial blood gas analysis							
$Paco_2$, Torr[c]	23	...	38.0 ± 0.7	12	...	39.0 ± 0.9	.40[d]
Pao_2, Torr[c]	23	...	60.6 ± 2.1	12	...	56.3 ± 3.0	.25[d]
A-aDO_2, Torr[e]	23	...	43.5 ± 2.4	12	...	46.2 ± 3.3	.51[d]
Disease severity score	23	...	3 (3-4)	12	...	3.5 (3-5)	.58[a]
GM-CSF autoantibody, μg/mL	23	...	22.8 (8.5-33.2)	12	...	23.1 (16.9-34.2)	.94[a]
Previous lung lavage (> 6 mo prior to study)			...			...	.22[b]
Yes	5	22		5	42		
No	18	78		7	58		

Thirty-five patients completed both the high-dose and low-dose period of GM-CSF inhalation therapy. A-aDO_2 = alveolar-arterial oxygen difference; AT = additional treatment; FR = free from additional treatment; GM-CSF = granulocyte-macrophage colony-stimulating factor; IQR = interquartile range (range from the 25th to the 75th percentiles of the distribution).

[a]Calculated using the Wilcoxon rank sum test.

[b]Calculated using the χ^2 test.

[c]Measured with patient in a supine position and breathing room air.

[d]Calculated using Student *t* test.

[e]Calculated using the following equation: A-aDO_2 = (PB − PH_2O) × FIO_2 − $Paco_2$/R + {$Paco_2$ × FIO_2 × (1 − R)/R} − Pao_2, where PB = barometric pressure measured by local observatories; PH2O = partial pressure of water vapor in inspired air (assumed to be 47 mm Hg); FIO_2 = fractional concentration of oxygen in dry gas (assumed to be 0.21); and R = respiratory quotient (assumed to be 0.8).

(e-Table 1). However, changes in A-aDO_2 during the GM-CSF treatment were significantly higher in the AT-GM group.

Association of Clinical Parameters With Requirement for Additional Treatment

There was no significant difference in baseline findings in terms of Pao_2, $Paco_2$, FEV_1, and DLCO between AT and FR groups. Both %VC (% predicted value) and %FVC were higher in the FR group ($P < .01$) (Fig 2A, Table 2, e-Fig 2). There was no correlation between baseline %VC and age ($P = .97$), sex ($P = .41$), baseline Pao_2 ($P = .18$), or baseline %DLCO ($P = .34$). There was no significant difference in high-resolution CT scan scores and serum markers, including LDH, KL-6, carcinoembryonic antigen (CEA), SP-A, and SP-D (Table 2).

As for differential blood cell counts, no significant difference was observed between FR and AT groups, except for numbers of basophils and platelets. The cell density of macrophages in BALF was lower in the FR group than those in the AT group ($P < .05$), whereas lymphocytes were lower in the AT group as compared with the FR group.

Next, clinical parameters at the end of treatment were evaluated. The %DLCO was lower in the AT group than that in the FR group, and serum markers (eg, LDH, KL-6, CEA, SP-D, SP-A) and CT scan scores were higher in the AT group than those in the FR group at the end of treatment ($P < .05$). However, there was no significant difference in A-aDO_2, blood cell counts, and cell differentials in BALF (Table 3). The patients free from additional treatment maintained the improved disease severity score initially achieved (e-Fig 3).

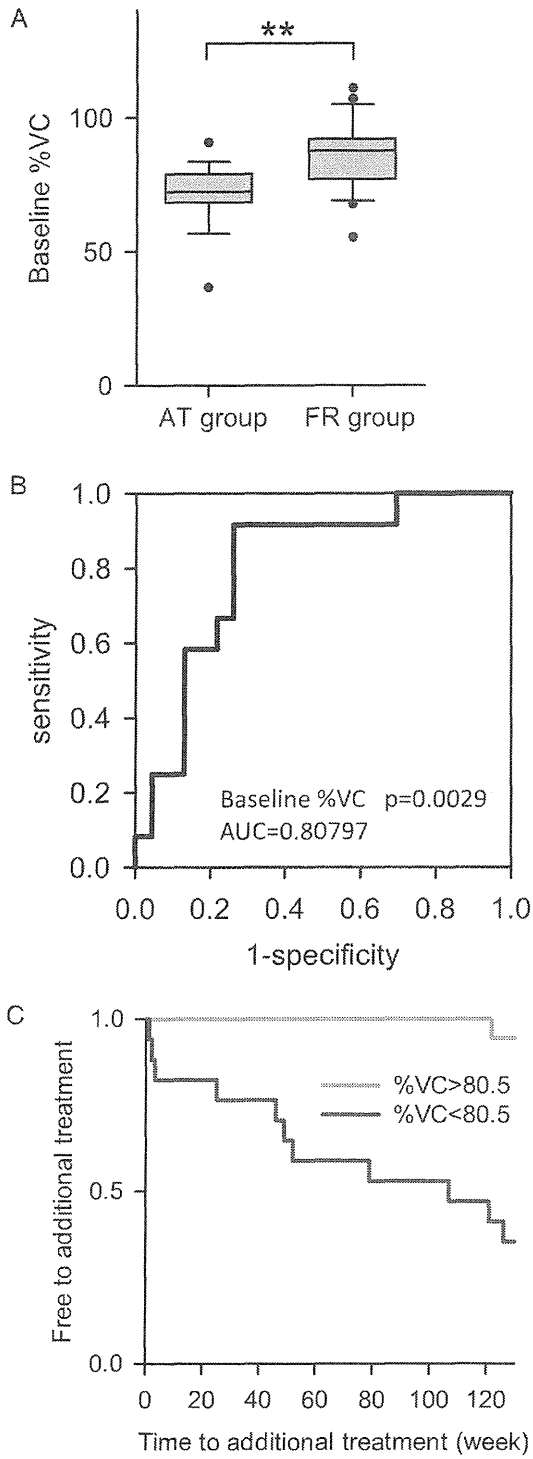

FIGURE 2. The association between VC (% predicted, %VC) and additional treatments during the 30-mo observation period (**$P < .01$). A, Baseline levels of %VC in FR and AT patient groups. B, Receiver operating curve of %VC. C, Kaplan-Meier plot showing patients of the high %VC group (%VC ≥ 80.5) and those of the low %VC group (%VC < 80.5). AUC = area under the receiver operating curve; VC = vital capacity. See Figure 1 legend for expansion of other abbreviations.

Predictive Value of VC for Prognosis After GM-CSF Inhalation

Because only %VC and %FVC differed between FR and AT groups among treatment-related pretreatment factors, the predictive value of parameters for recurrence after GM-CSF inhalation was evaluated using receiver operating characteristics curve (ROC) analysis and Kaplan-Meier analysis of time to additional treatment.

For ROC analysis, the area under the ROC curve was calculated nonparametrically, as proposed by Hanley and McNeil.[22] An additional therapy was defined as a positive indicator for disease recurrence. When the cutoff level of 80.5% was set for %VC, the baseline %VC predicted the additional therapy with a sensitivity of 92% and a specificity of 74% (Fig 2B).

For Kaplan-Meier analysis of time to additional treatment, we divided the patients into two groups, namely the high %VC group (%VC ≥ 80.5) and the low %VC group (%VC < 80.5). A significant difference in the time to additional treatment between the two groups was seen when the whole period of follow-up was compared ($P = .0001$) (Fig 2C). In the univariate Cox proportional analysis of baseline markers, %VC < 80.5% (hazard ratio, 18.42; 95% CI, 3.55-337.68; $P < .0001$) was associated with additional treatment, whereas no correlations were found between additional treatment and age, sex, baseline PaO_2, changes in A-aDO_2, and baseline levels of LDH, KL-6, SP-A, CEA, and anti-GM-CSF-Ab.

Subgroup Analysis: To test whether VC is an independent predictive factor for the time to additional therapy, we did subgroup analyses because of the small number of the AT patients. The patients were divided into two groups of an upper one-half and a lower one-half regarding age; sex; baseline PaO_2; change in A-aDO_2; baseline levels of LDH, KL-6, SP-A, CEA; and anti-GM-CSF-Ab. In these subgroups, a significant difference in the time to additional treatment between the high %VC group (%VC ≥ 80.5) and the low %VC group (%VC < 80.5) was still evident, suggesting that VC might be an independent factor predicting the time to additional therapy (e-Fig 4).

Time Course of Autoantibody Levels: In our previous reports, serum levels of anti-GM-CSF-Ab levels did not change during treatment.[16] To study longitudinal changes of serum levels of anti-GM-CSF-Ab after the inhaled GM-CSF therapy, serum samples were collected for anti-GM-CSF-Ab testing as an optional evaluation after the 30-month observation period. The serum levels were unchanged during the observation period except for three cases (e-Fig 5). In two cases, the serum levels increased by > 100 μg/mL, and one case required an additional treatment, whereas

Table 2—*Baseline Pulmonary Function, Radiologic Appearance, Serum Biomarkers, Hematologic Indexes, and BALF Cell Findings in Patients With PAP in FR and AT Groups Before GM-CSF Inhalation Treatment*

Measure	FR		AT		*P* Value
	No.	Mean ± SE or Median (IQR)	No.	Mean ± SE or Median (IQR)	
Pulmonary function					
VC, % predicted	23	85.9 ± 2.7	12	71.6 ± 3.8	.0045[a]
FVC, % predicted	23	85.3 ± 2.8	12	71.4 ± 3.9	.0064[a]
FEV_1/FVC	23	87.1 ± 2.0	12	84.9 ± 2.7	.51[a]
DLCO, % predicted	23	57.0 ± 3.4	10	46.0 ± 5.1	.082[a]
HRCT scan scores[b]					
Upper lung region	23	3 (2-5)	12	4.5 (2-5)	.12[c]
Middle lung region	23	4 (3-5)	11	4 (3-5)	.38[c]
Lower lung region	23	4 (3-5)	12	5 (4-5)	.36[c]
Serum biomarkers of PAP					
LDH, IU/L	23	287 ± 19	12	325 ± 26	.26[a]
CEA, ng/mL	23	6.2 ± 1.0	12	8.0 ± 1.4	.30[a]
KL-6, U/L	23	10,038 ± 1,531	12	9,434 ± 2,120	.81[a]
SP-A, ng/mL	23	127 ± 15	12	153 ± 20	.29[a]
SP-D, ng/mL	23	227 ± 25	12	290 ± 34	.14[a]
Hematologic indexes					
WBC count, cells/μL	23	5,608 ± 267	12	6,358 ± 370	.11[a]
Neutrophils, cells/μL	22	3,428 ± 200	12	3,596 ± 271	.62[a]
Monocytes, cells/μL	22	344 ± 21	12	396 ± 28	.15[a]
Lymphocytes, cells/μL	22	1,730 ± 147	12	2,122 ± 198	.12[a]
Eosinophils, cells/μL	22	107 ± 28	12	199 ± 38	.058[a]
Basophils, cells/μL	22	18.3 ± 4.3	12	45.3 ± 5.9	.0008[a]
Hemoglobin, g/dL	23	15.4 ± 0.3	12	14.4 ± 0.4	.058[a]
Platelets, $\times 10^3$ cells/μL	23	224 ± 9.1	11	271 ± 13	.0046[a]
BALF cell classification, %					
Alveolar macrophages	17	63 ± 3.6	5	38 ± 6.7	.0036[a]
Neutrophils	17	5.2 ± 1.5	5	10.8 ± 2.7	.082[a]
Eosinophils	17	0.84 ± 0.32	5	0.40 ± 0.60	.52[a]
Lymphocytes	17	31.2 ± 3.8	5	50.4 ± 7.1	.027[a]

BALF = BAL fluid; CEA = carcinoembryonic antigen; DLCO = diffusing capacity of the lung for carbon monoxide; HRCT = high-resolution CT; KL-6 = Krebs von den Lungen-6; LDH = lactate dehydrogenase; PAP = pulmonary alveolar proteinosis; SP = surfactant protein; VC = vital capacity. See Table 1 legend for expansion of other abbreviations.

[a]Calculated using Student *t* test.

[b]Described previously.[18] left lung.

[c]Calculated using the Wilcoxon rank sum test.

the others did not. In the third case, the serum levels decreased to 0.47 μg/mL, and additional treatments were not required.

Discussion

In the present study we have prospectively analyzed, for the time to our knowledge, the requirements of additional therapy and disease severity scores in 35 patients who completed GM-CSF inhalation therapy. The results demonstrate that 23 patients were free from administration of additional treatment during the 30-month observation period, indicating the enduring nature of the therapy. VC could be a useful predictive parameter for the recurrence of disease after GM-CSF therapy. This study contributes to the promotion of GM-CSF inhalation for initial therapy of aPAP.

WLL remains the standard of care today. A retrospective analysis of 231 cases found clinically significant improvement in Pa_{O_2}, FEV_1, VC, and DLCO and reported that the median duration of clinical benefit from lavage was 15 months.[2] In a report of 21 patients with PAP who underwent WLL in an experienced center, >70% of patients remained free from recurrent PAP during 7-year observation.[23] In our study, the median time to application of additional therapy was 30 months after GM-CSF therapy, suggesting the effects of GM-CSF inhalation may be comparable to those of WLL. Notably, the difference in changes in $A\text{-}aDO_2$ during the GM-CSF treatment between the AT-WLL group patients and the AT-GM group patients suggests that nonresponders to the first GM-CSF treatment might be likely to undergo WLL when disease recurred.

In a single-center, phase 2 study for subcutaneous administration of GM-CSF for PAP, Venkateshiah et al[12]

Table 3—*Pulmonary Function, Radiologic Appearance, Serum Biomarkers, Hematologic Indexes, and BALF Cell Findings in Patients With PAP in FR and AT Groups at the End of GM-CSF Inhalation Treatment and Before the 30-Mo Observation*

Measure	FR		AT		*P* Value
	No.	Mean ± SE or Median (IQR)	No.	Mean ± SE or Median (IQR)	
Pulmonary function					
VC, % predicted	23	93.4 ± 3.0	12	74.2 ± 4.2	.0007[a]
FVC, % predicted	23	80.5 ± 3.3	12	72.2 ± 4.5	.0025[a]
FEV_1/FVC	23	85.6 ± 1.6	12	84.7 ± 2.2	.73[a]
DLCO, % predicted	23	68.4 ± 3.4	11	46.8 ± 4.7	.0006[a]
HRCT scan scores[b]					
Upper lung region	23	2 (2-3)	12	3.5 (2-4)	.036[c]
Middle lung region	23	3 (2-3)	12	4 (2.25-4.75)	.023[c]
Lower lung region	23	2 (2-3)	12	4 (2.25-5)	.0039[c]
Serum biomarkers of PAP					
LDH, IU/L	23	242 ± 13	12	308 ± 18	.0064[a]
CEA, ng/mL	23	2.7 ± 0.6	12	5.7 ± 0.8	.0075[a]
KL-6, U/L	23	3,675 ± 735	12	6,565 ± 1,017	.028[a]
SP-A, ng/mL	23	80 ± 12	12	131 ± 16	.015[a]
SP-D, ng/mL	23	170 ± 34	12	304 ± 47	.027[a]
Hematologic indexes					
WBC count, cells/μL	23	5,213 ± 306	12	5,797 ± 424	.27[a]
Neutrophils, cells/μL	22	2,961 ± 205	12	3,026 ± 277	.85[a]
Monocytes, cells/μL	22	320 ± 30	12	338 ± 41	.74[a]
Lymphocytes, cells/μL	22	1,755 ± 131	12	2,153 ± 177	.080[a]
Eosinophils, cells/μL	22	145 ± 40	12	233 ± 55	.20[a]
Basophils, cells/μL	22	27.4 ± 5.9	12	43.7 ± 8.4	.12[a]
Hemoglobin, g/dL	23	14.8 ± 1.3	12	14.4 ± 1.4	.52[a]
Platelets, × 10^3 cells/μL	23	214 ± 9.0	12	235 ± 12	.17[a]
BALF cell classification, %					
Alveolar macrophages	13	67 ± 4.1	5	58 ± 6.7	.28[a]
Neutrophils	13	6.6 ± 2.2	5	7.4 ± 3.5	.86[a]
Eosinophils	13	0.90 ± 0.46	5	0.82 ± 0.75	.93[a]
Lymphocytes	13	25.6 ± 4.8	5	33.2 ± 7.7	.41[a]

See Table 1 and 2 legends for expansion of abbreviations.
[a]Calculated using Student *t* test.
[b]Described previously,[15] left lung.
[c]Calculated using the Wilcoxon's rank sum test.

reported that nine of 21 patients (43%) required WLL. In a retrospective study of 12 patients who underwent aerosolized GM-CSF therapy, Wylam et al[17] reported that five of 11 responders had recurrence of disease. In four of five patients, the mean time to relapse was 6.3 months and ranged from 5.5 to 12 months.[15] It is notable that the dose of GM-CSF used in their study was twice that used in our study, although the prognosis of our cases was comparable to that of their study.

PAP is often described as a lung disorder with restrictive physiology. In the present study, 18 of 35 patients were in the normal range (≤ 80) in %FVC, whereas the other 17 patients were mildly to moderately restricted, which was comparable to previous studies.[24] Seymour et al[25] investigated 14 patients who underwent subcutaneous GM-CSF administration and suggested that higher VC before treatment was one marker to define responsiveness to GM-CSF therapy. In the present study, VC did not correlate with responsiveness to GM-CSF therapy, but it showed significant association with the requirement for additional treatment. Although limited by the small number of cases, the subgroup analyses suggested that VC is an independent factor from age, sex, baseline PaO_2, change in $A\text{-}aDO_2$, and baseline levels of serum markers, including anti-GM-CSF-Ab. However, there is a possibility that some clinical variables might be intrinsically related to VC. The physicians' decision for retreatment might be influenced by such clinical markers. Notably, a recent study of a series of patients with PAP followed in a reference center reported that the need for lavage was significantly associated with FVC.[26]

Reduction of VC might be due to two different factors: accumulation of surfactant-derived materials in the alveolar space and fibrotic changes of lung tissue. In a study of a quantitative CT scan analysis of patients with PAP who underwent WLL and showed improvements in %DLCO and %FVC, Perez et al[27] demonstrated that there was a reduction in lung weight

following lavage, which correlated with the dry weight of the lavage effluent. The study demonstrated a shift in the regional lung inflation toward more inflated lung with a corresponding increase in the mean lung inflation. Surfactant accumulation might be associated with an elevated ventilation-perfusion mismatch and disproportionately impaired DLCO in patients with aPAP.[2] Seymour et al[25] demonstrated serum levels of SP-A correlated with VC in 14 patients at baseline. The present study also showed that serum levels of SP-A correlated with VC at baseline as well as after treatment. However, requirement of additional therapy was not significantly associated with SP-A at baseline. Surfactant materials might be easily redistributed in alveolar spaces and may not be related to the impairment of lung tissue that might lead to additional treatment.

The other factor, fibrotic changes of lung tissue, might be maintained even after GM-CSF therapy or WLL. Pulmonary fibrosis has been reported to be associated with PAP, and exposure to oxygen or repeated WLL have been suggested as potential contributors to fibrosis. Although irreversible scarring of the lung is rarely associated with PAP, a small fraction of patients with PAP demonstrated substantially impaired %VC and rather poor prognosis. To investigate this possibility, two radiologists reevaluated baseline CT scans of 32 of the 35 participants for findings other than PAP without knowing the study results regarding responsiveness and prognosis of the GM-CSF inhalation. They only pointed out traction bronchiectasis in one patient (responder, FR), bronchiectasis in one patient (responder, FR), and multiple bullae in one patient (responder, AT). Thus, we failed to find any significant association between fibrotic change in CT scan and requirement of additional treatments. In the present study, the mean %VC levels of patients in the FR group improved from 85.9% to 93.4%, whereas those of patients in the AT group changed from 71.6% to 74.2%. The difference in improvement between the groups might be associated with the balance of surfactant accumulation and lung fibrosis in the lungs of patients.

For future studies, it would be useful to explore novel treatment regimens for patients with moderately impaired VC. As shown in this study, inhaled GM-CSF therapy did not change serum levels of anti-GM-CSF-Ab. However, the BALF titers of anti-GM-CSF-Ab were reduced in responders, which was likely due to the improved clearance in alveolar spaces. The future treatments might include a combination of GM-CSF inhalation with WLL to improve the environment of airway/alveolar spaces or with administration of rituximab to reduce the systemic production of anti-GM-CSF-Ab.

In conclusion, this study demonstrated that VC might be clinically useful in predicting the need for additional therapy in patients with aPAP who were treated with inhaled GM-CSF therapy. We believe this study contributes to improving the quality of life and treatments for patients with aPAP.

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Additional information: The e-Figures and e-Table can be found in the "Supplemental Materials" area of the online article.

References

1. Rosen SH, Castleman B, Liebow AA. Pulmonary alveolar proteinosis. *N Engl J Med*. 1958;258(23):1123-1142.
2. Seymour JF, Presneill JJ. Pulmonary alveolar proteinosis: progress in the first 44 years. *Am J Respir Crit Care Med*. 2002;166(2):215-235.
3. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N Engl J Med*. 2003;349(26):2527-2539.
4. Kitamura T, Tanaka N, Watanabe J, et al. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J Exp Med*. 1999;190(6):875-880.
5. Uchida K, Nakata K, Trapnell BC, et al. High-affinity autoantibodies specifically eliminate granulocyte-macrophage colony-stimulating factor activity in the lungs of patients with idiopathic pulmonary alveolar proteinosis. *Blood*. 2004;103(3):1089-1098.
6. Dranoff G, Crawford AD, Sadelain M, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science*. 1994;264(5159):713-716.
7. Stanley E, Lieschke GJ, Grail D, et al. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci U S A*. 1994;91(12):5592-5596.
8. Sakagami T, Beck D, Uchida K, et al. Patient-derived granulocyte/macrophage colony-stimulating factor autoantibodies reproduce pulmonary alveolar proteinosis in nonhuman primates. *Am J Respir Crit Care Med*. 2010;182(1):49-61.
9. Seymour JF, Dunn AR, Vincent JM, Presneill JJ, Pain MC. Efficacy of granulocyte-macrophage colony-stimulating factor in acquired alveolar proteinosis. *N Engl J Med*. 1996;335(25):1924-1925.
10. Seymour JF, Presneill JJ, Schoch OD, et al. Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. *Am J Respir Crit Care Med*. 2001;163(2):524-531.
11. Kavuru MS, Sullivan EJ, Piccin R, Thomassen MJ, Stoller JK. Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis. *Am J Respir Crit Care Med*. 2000;161(4 pt 1):1143-1148.
12. Venkateshiah SB, Yan TD, Bonfield TL, et al. An open-label trial of granulocyte macrophage colony stimulating factor therapy for moderate symptomatic pulmonary alveolar proteinosis. *Chest*. 2006;130(1):227-237.
13. Price A, Manson D, Cutz E, Dell S. Pulmonary alveolar proteinosis associated with anti-GM-CSF antibodies in a child: successful treatment with inhaled GM-CSF. *Pediatr Pulmonol*. 2006;41(4):367-370.
14. Schoch OD, Schanz U, Koller M, et al. BAL findings in a patient with pulmonary alveolar proteinosis successfully treated with GM-CSF. *Thorax*. 2002;57(3):277-280.
15. Anderson PM, Markovic SN, Sloan JA, et al. Aerosol granulocyte macrophage-colony stimulating factor: a low toxicity, lung-specific biological therapy in patients with lung metastases. *Clin Cancer Res*. 1999;5(9):2316-2323.
16. Tazawa R, Hamano E, Arai T, et al. Granulocyte-macrophage colony-stimulating factor and lung immunity in pulmonary alveolar proteinosis. *Am J Respir Crit Care Med*. 2005;171(10):1142-1149.
17. Wylam ME, Ten R, Prakash UB, Nadrous HF, Clawson ML, Anderson PM. Aerosol granulocyte-macrophage colony-stimulating factor for pulmonary alveolar proteinosis. *Eur Respir J*. 2006;27(3):585-593.
18. Tazawa R, Trapnell BC, Inoue Y, et al. Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis. *Am J Respir Crit Care Med*. 2010;181(12):1345-1354.
19. Inoue Y, Trapnell BC, Tazawa R, et al. Characteristics of a large cohort of autoimmune pulmonary alveolar proteinosis in Japan. *Am J Respir Crit Care Med*. 2008;177(7):752-762.
20. Ohashi K, Sato A, Takada T, et al. Direct evidence that GM-CSF inhalation improves lung clearance in pulmonary alveolar proteinosis. *Respir Med*. 2012;106(2):284-293.
21. Uchida K, Beck DC, Yamamoto T, et al. GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. *N Engl J Med*. 2007;356(6):567-579.
22. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*. 1982;143(1):29-36.
23. Beccaria M, Luisetti M, Rodi G, et al. Long-term durable benefit after whole lung lavage in pulmonary alveolar proteinosis. *Eur Respir J*. 2004;23(4):526-531.
24. Bonella F, Bauer PC, Griese M, Ohshimo S, Guzman J, Costabel U. Pulmonary alveolar proteinosis: new insights from a single-center cohort of 70 patients. *Respir Med*. 2011;105(12):1908-1916.
25. Seymour JF, Doyle IR, Nakata K, et al. Relationship of anti-GM-CSF antibody concentration, surfactant protein A and B levels, and serum LDH to pulmonary parameters and response to GM-CSF therapy in patients with idiopathic alveolar proteinosis. *Thorax*. 2003;58(3):252-257.
26. Campo I, Mariani F, Rodi G, et al. Assessment and management of pulmonary alveolar proteinosis in a reference center. *Orphanet J Rare Dis*. 2013;8:40.
27. Perez A IV, Coxson HO, Hogg JC, Gibson K, Thompson PF, Rogers RM. Use of CT morphometry to detect changes in lung weight and gas volume. *Chest*. 2005;128(4):2471-2477.

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RESEARCH ARTICLE Open Access

Secondary pulmonary alveolar proteinosis complicating myelodysplastic syndrome results in worsening of prognosis: a retrospective cohort study in Japan

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Abstract

Background: Secondary pulmonary alveolar proteinosis (sPAP) is a very rare lung disorder comprising approximately 10% of cases of acquired PAP. Hematological disorders are the most common underlying conditions of sPAP, of which 74% of cases demonstrate myelodysplastic syndrome (MDS). However, the impact of sPAP on the prognosis of underlying MDS remains unknown. The purpose of this study was to evaluate whether development of sPAP worsens the prognosis of MDS.

Methods: Thirty-one cases of sPAP and underlying MDS were retrospectively classified into mild and severe cases consisting of very low-/low-risk groups and intermediate-/high-/very high-risk groups at the time of diagnosis of MDS, according to the prognostic scoring system based on the World Health Organization classification. Next, we compared the characteristics, disease duration, cumulative survival, and prognostic factors of the groups.

Results: In contrast to previous reports on the prognosis of MDS, we found that the cumulative survival probability for mild MDS patients was similar to that in severe MDS patients. This is likely due to the poor prognosis of patients with mild MDS, whose 2-year survival rate was 46.2%. Notably, 75% and 62.5% of patients who died developed fatal infectious diseases and exacerbation of PAP, respectively, suggesting that the progression of PAP *per se* and/or PAP-associated infection contributed to poor prognosis. The use of corticosteroid therapy and a diffusing capacity of the lung for carbon monoxide of less than 44% were predictive of poor prognosis.

Conclusion: Development of sPAP during the course of MDS may be an important adverse risk factor in prognosis of patients with mild MDS.

Keywords: Proteinosis, Myelodysplastic syndrome, GM-CSF, WPSS, Secondary pulmonary alveolar proteinosis, MDS, PAP, Refractory anemia

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Background

Pulmonary alveolar proteinosis (PAP), a rare disorder predominantly affecting the lungs, is characterized by accumulation of surfactant lipids and proteins in the alveoli and terminal bronchioles [1]. PAP is clinically classified into three distinct forms, namely, autoimmune, secondary, and congenital PAP [2]. Autoimmune PAP is associated with disruption of granulocyte/macrophage colony-stimulating factor (GM-CSF) signaling caused by high levels of GM-CSF autoantibody in the lungs [3]. Secondary PAP (sPAP) results from underlying diseases that presumably impair surfactant clearance because of abnormal numbers and functions of alveolar macrophages (AMs). Of the 40 cases of sPAP previously reported by our group [4], 88% (n = 35) involved hematological disorders as underlying diseases. The probability of survival at two years was 46% in cases with sPAP complicating hematological disorders. The median survival time for all cases including 17 patients who died within two years of the sPAP diagnosis was 16 months. Although myelodysplastic syndrome (MDS) is the most frequent underlying disease of sPAP (n = 26, 65%), little information is available on the prognostic impact of development of sPAP on patient outcome.

MDS, a group of clonal hematological stem-cell disorders with ineffective myeloid hematopoiesis and varying degrees of bone marrow failure, is associated with a significant risk of progression to acute myeloid leukemia (AML). Clinical manifestations are variable, from indolent conditions with near-normal life expectancy to forms that rapidly develop into AML [5]. Clarifying much of this heterogeneity, the World Health Organization (WHO) developed a classifications of MDS based on the presence of unilineage or multilineage dysplasia, bone marrow blast cell count, and cytogenetic features: refractory anemia (RA), RA with ringed sideroblasts (RARS), refractory cytopenia with multilineage dysplasia (RCMD), RCMD with ringed sideroblasts (RCMD-RS), RA with excess of blasts-1 (RAEB-1), and RA with excess of blasts-2 (RAEB-2) [6]. In 2007, Malcovati *et al.* developed a new prognostic scoring system (WHO classification-based prognostic scoring system (WPSS)) according to WHO subgroup, karyotype, and transfusion requirement. Through this system, cases with MDS are classified into very low-, low-, intermediate-, high-, or very high-risk groups [7]. WPSS is a dynamic system that accurately predicts the survival and risk of leukemic evolution in MDS patients at any time during the course of their disease. This time-dependent system seems particularly useful for lower-risk patients and for implementing risk-adapted treatment strategies.

Given the validation of WPSS criteria as a prognostic indicator for the course of MDS, it is applicable in prognosis evaluation of MDS complicated by PAP (MDS/sPAP). In the present study, we evaluated this issue for the first time and found that development of sPAP worsens the prognosis of patients with otherwise low-risk MDS.

Methods

Subjects

Thirty-one patients in Japan who were diagnosed with sPAP with underlying MDS from July 1999 to June 2013 were evaluated. We obtained agreement from all treating physicians for each identified case, according to the Guidelines for Epidemiological Studies by The Ministry of Health, Labour, and Welfare. This was a retrospective cohort study approved by the Ethical Board of Kyorin University (H23-085-01). Cases, part of which were reported previously, were identified retrospectively [8-18].

Diagnosis

Diagnosis of MDS was made according to the 2002 WHO criteria [6]. One patient with unclassified MDS, two patients with myelofibrosis, and two patients with 20% marrow blasts who were considered as having AML were excluded from the study. MDS with isolated chromosome 5 deletion (del(5q)) and marrow blasts of <5% were included. Next, the patients were classified into RA, RARS, RCMD, RAEB-1, and RAEB-2 groups. Karyotypes were classified by using the International System for Cytogenetic Nomenclature Criteria [19]. Diagnosis of PAP was based on the following criteria: 1. histopathological findings from specimens obtained by surgical biopsy or transbronchial lung biopsy, or milk-like appearance with typical cytological findings from bronchoalveolar lavage fluid (BALF); 2. typical high-resolution computed tomography (HRCT) findings for PAP, such as ground glass opacity, consolidation, and interlobular septal thickening; and 3. negative result for serum GM-CSF autoantibody.

Classification by WPSS

The WPSS score was calculated according to the method of Malcovati *et al.* [7] The score was calculated from the data of WHO subgroups (RA/RARS/5q−, RCMD/RCMD-RS, RAEB-1, and RAEB-2), karyotype abnormalities categorized according to the International Prognostic Scoring System [20], and transfusion requirement. The same weight (score of ≥1) was assigned to each variable for WHO subgroup, karyotype, and transfusion requirement. Based on the score, patients were classified according to the following five risk groups: very low (score = 0), low (score = 1), intermediate (score = 2), high (score = 3 to 4), and very high (score = 5 to 6). Transfusion dependency was defined as having at least one

red blood cell (RBC) transfusion every eight weeks over a period of four months.

Statistical analysis

The cumulative probability of survival and risk of progression to leukemia were estimated by using the Kaplan–Meier method. Patients undergoing transplantation treatment were censored at the time of the procedure in order to exclude any potential source of bias due to differential treatment. Variable data were analyzed through Kaplan–Meier methods to estimate the cumulative probabilities of overall survival. The difference in the cumulative probabilities within subcategories of patients was compared by using two-sided log rank test. Survival analyze was performed using Cox proportional model with time-dependent covariates to assess the effect of the variables of interest on overall survival.

Numeric data were evaluated for normal distribution and for equal variance by using the Kolmogorov–Smirnov test and Levene's median test, respectively. Nonparametric data are presented as medians. Categorical data are presented as a percentage of the total or numerically, as appropriate. Statistical comparisons of nonparametric data were compared through the Wilcoxon test. Comparisons of categorical data were made with chi-square or Fischer's exact tests. All tests were two-sided. Statistical significance was indicated by p values of <0.05. Data were analyzed by using SPSS 17.0 software for Windows.

Results

From September 1999 to May 2013, we centrally analyzed for GM-CSF autoantibody in the sera of 619 Japanese cases that had been diagnosed as PAP. Of those cases, 561 were positive for the antibody and 58 were negative. In the cases negative for GM-CSF antibody, 51 demonstrated obvious underlying diseases such as hematological disorders, autoimmune diseases, and infectious diseases. As shown in Figure 1, hematological disorders were the most common underlying disease, 31 cases of which involved MDS. These cases were investigated retrospectively in this study.

Demographic data are shown in Table 1. Diagnosis of MDS was performed in accordance with WHO criteria; 19, 1, 5, 3, and 3 cases were RA, RARS, RCMD, RAEB-1, and RAEB-2, respectively. Karyotyping revealed 2 cases of "high-riskes of g e, 24 cases of "intermediate-riskmediatef, and 5 cases of "low-risks disease. As previously reported [10], there was over-representation of trisomy 8, as it was present in 16 cases (51.6%). RBC transfusion dependency was observed in 11 cases.

PAP was diagnosed on the basis of the BALF and HRCT results in 23 cases and by surgical biopsy and HRCT in eight cases. In 23 cases, diagnosis of MDS was done before diagnosis of PAP, whereas eight cases were diagnosed simultaneously.

According to the WPSS, the cases were classified into 2, 11, 7, 9, and 2 cases of very low-, low-, intermediate-, high-, and very high-risk groups, respectively. For statistical analysis, very low-/low-risk groups and intermediate-/high-/very high-risk groups were categorized as "mild MDS" and "severe MDS" DSere iz, respectively (Table 1). There was no difference in sex and age at the diagnosis of MDS and in hemoglobin concentration, absolute neutrophil count, and platelet count between mild and severe MDS cases. Then, the median age at diagnosis of

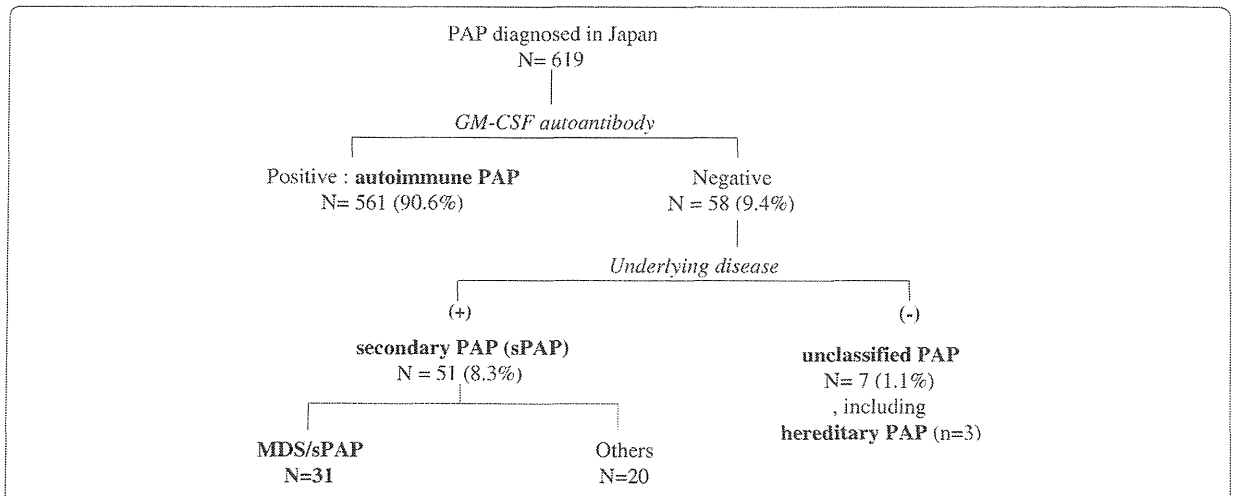

Figure 1 Disposition of patients with pulmonary alveolar proteinosis (PAP) who were enrolled into this study. Participants were stratified according to the presence or absence of granulocyte/macrophage-colony stimulating factor (GM-CSF) autoantibody, and then according to the presence or absence of an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

Table 1 Demographic data at diagnosis of MDS in each group classified according to the WPSS

Median (min.–max.)	Total	<WPSS risk groups>		p value
		<Very low + low >	<Inter – +high + very high>	
		Mild MDS	Severe MDS	
	(n = 31)	(n = 13)	(n = 18)	
Sex (M/F)	19/12	7/6	12/6	0.71
Age at Dx of MDS	50 (27–75)	45 (30–67)	50 (27–75)	0.54
HbG (g/dl)	9.4 (4.8–16.4)	11.4 (5.5–16.4)	9.0 (4.8–13.9)	0.06
ANC ($\times 10^9$/L)	1.84 (0.01–7.54)	1.46 (0.45–6.97)	2.74 (0.01–7.54)	0.68
PLT ($\times 10^9$/L)	65 (6–219)	45 (14–219)	69 (6–196)	0.31
WHO subgroup: n (%)				
RA/RARS	20 (65)	13 (100)	7 (39)	<0.001
RCMD	5 (16)	0 (0)	5 (28)	0.058
RAEB-1,2	6 (19)	0 (0)	6 (33)	0.02
Karyotype*: n (%)				
Good type	2 (7)	2 (15)	0	0.16
Intermediate type	24 (77)	11 (85)	13 (72)	0.66
Poor type	5 (16)	0 (0)	5 (28)	0.058
RBC transfusion dependency**: n (%)	11 (35)	0 (0)	11 (61)	<0.001

(*) Cytogenetics was as follows. Good type: normal, –Y, del(5q), del(20q); poor type: complex (≥ three abnormalities), chromosome 7 anomalies; and intermediate type: other abnormalities.
(**) RBC transfusion dependency was defined as having at least one RBC transfusion every eight weeks over a period of four months. ANC, absolute neutrophil count; Dx, diagnosis; HbG, hemoglobin; MDS, myelodysplastic syndrome; PLT, platelets; RA, refractory anemia; RAEB, refractory anemia with blasts; RARS, refractory anemia with ringed sideroblasts; RBC, red blood cell; RCMD, refractory anemia with multilineage dysplasia; WHO, World Health Organization; WPSS, WHO classification-based prognostic scoring system.

Table 2 Clinical status at death (n = 17)

	No.	WPSS at diagnosis	WHO criteria at diagnosis of MDS	AML progression	Progression of PAP	Pneumonia
				6 (35.3%)	11 (64.7%)	11 (64.7%)
Mild MDS	1	Very low + low	RA			•
	2		RA		•	
	3		RA	•		•
	4		RA		•	•
	5		RA	•		
	6		RA		•	
	7		RA		•	
Severe MDS	8	Intermediate	RA			•
	9		RA		•	•
	10		RA		•	•
	11		RA	•	•	•
	12		RA	•		•
	13		RCMD		•	
	14	High + very high	RA		•	•
	15		RCMD	•	•	
	16		RAEB-1		•	•
	17		RAEB-2	•		•

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; PAP, pulmonary alveolar proteinosis; RA, refractory anemia; RAEB, refractory anemia with blasts; RCMD, refractory anemia with multilineage dysplasia; WHO, World Health Organization; WPSS, WHO classification-based prognostic scoring system.

MDS/sPAP was 51 years, and 84% of cases were symptomatic, with the most common symptoms being fever (45%), dyspnea on effort (42%), and cough (42%). The value of serum Krebs von den Lungen-6 (KL-6) and surfactant protein-D (SP-D) were elevated, and the diffusing capacities of the lung for carbon monoxide (% DLco) were very low in the absence of ventilation disorder in pulmonary function tests. There was no difference in the frequency of respiratory symptoms between patients with mild MDS and those with severe MDS. Serum biomarkers and pulmonary function tests showed no significant difference between the two groups.

Follow-up periods after diagnosis of MDS ranged from five to 254 months (median, 40 months) in all patients (Additional file 1: Figure S1 and Additional file 2: Figure S2). During the follow-up period, 7 patients with mild MDS and 10 patients with severe MDS died, and 2 patients with mild MDS and 4 patients with severe MDS progressed to AML (Table 2). Two and five patients in the mild MDS and severe MDS groups, respectively, underwent transplantation therapies. They were censored at the time of the procedure. The duration from diagnosis of MDS to diagnosis of sPAP was variable, ranging from 0 to 168 months in the mild MDS group and from 0 to 228 months in the severe MDS group (Figure 2A). The median duration of MDS prior to diagnosis of sPAP in the mild MDS group was significantly longer than that in the severe MDS group

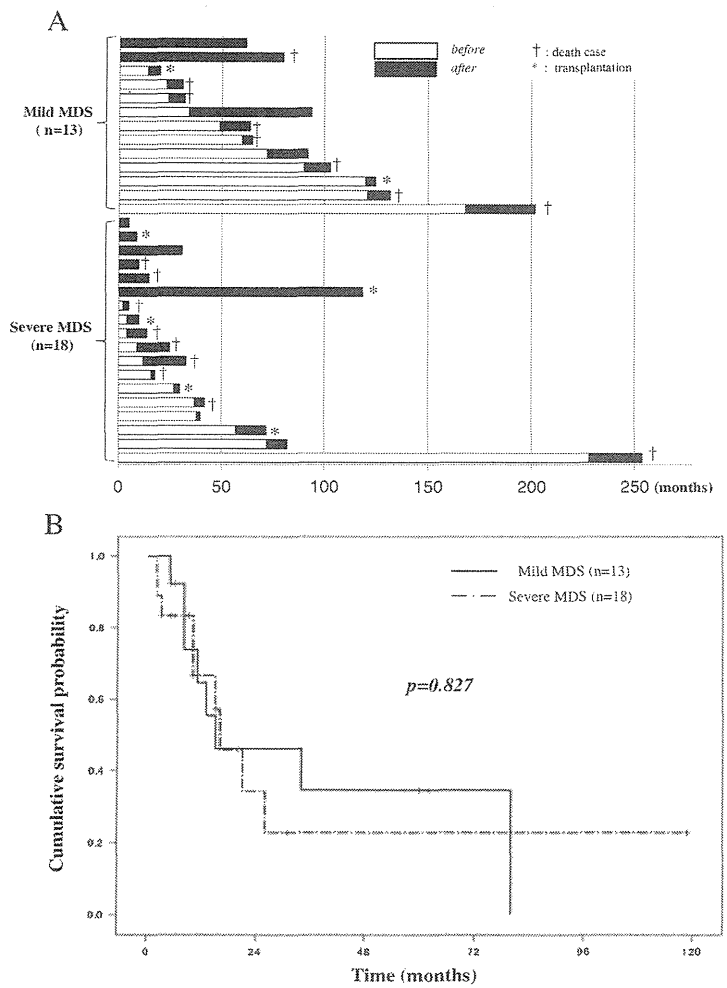

Figure 2 Disease duration of secondary PAP in patients with MDS. A: Duration of disease before or after the diagnosis of secondary pulmonary alveolar proteinosis (sPAP). The horizontal axis indicates the month after the diagnosis of myelodysplastic syndrome. The open column and closed column indicate the duration of disease respectively before and after the diagnosis of sPAP. The case that resulted in death is marked by † and the case that underwent transplantation therapy is marked by *. **B**: Cumulative survival probability after diagnosis of secondary PAP (sPAP) in patient groups with mild myelodysplastic syndrome (MDS) (n = 13) and severe MDS (n = 18). The horizontal axis indicates the month after the diagnosis of sPAP, and the vertical axis indicates the cumulative survival probability.

(p = 0.034). Three patients in the mild MDS group and one in the severe MDS group survived for more than five years after diagnosis of sPAP. Of those, spontaneous remission of sPAP occurred in three cases.

A previous report [7] demonstrated that prediction of survival was dependent on the severity of MDS (as defined by WPSS) at any time of the disease. In contrast, patients with mild MDS in our study had cumulative survival probability similar to that of patients with severe MDS (Figure 2B, p = 0.827). This similarity may be due to poor prognosis of mild MDS after diagnosis of PAP. The cumulative survival probability curves of mild and severe MDS groups with median survivals of 13 and 15 months, respectively, are comparable. Concerning causes for the death of seven patients in the mild MDS group were progression to AML in two cases, PAP exacerbation in four cases, and fatal infectious disease in three cases (Table 2). Concerning causes for the death of 10 patients in the severe MDS group were progression to AML in 4 cases, PAP exacerbation in 7 cases, and fatal infectious disease in 8 cases. Fatal infectious diseases consistently arose from severe pneumonia with (n = 4) or without systemic sepsis, suggesting that progression of PAP was the major cause of death in both mild and severe MDS patients. These results suggest that occurrence of sPAP principally reduced the survival of patients with mild MDS. Pathogens isolated in the fatal cases were identified as *Aspergillus* species (four cases), *Pseudomonas aeruginosa* (four cases), and non-tuberculosis *Mycobacterium* species (four cases).

By performing univariate analysis using Cox proportional model we then searched for potential prognostic factors. Age, sex, respiratory symptoms, history of respiratory failure, diagnostic procedure for sPAP, and MDS group (mild or severe), were not associated with survival at the time of diagnosis of sPAP (Table 3). Treatment with

Table 3 Univariate analysis of overall survival after diagnosis of sPAP in MDS

Variable at diagnosis of sPAP	(n)	75% of OS (months)	50% of OS (months)	HR (95% CI)	*P* value
Age: 51 yrs or younger	16	8	16		
Older than 52 yrs	15	10	15	1.29 (0.48-3.41)	0.607
Gender: Male	19	10	16		
Female	12	11	21	1.12 (0.43-2.94)	0.804
MDS group: mild	13	10	15		
severe	18	11	16	1.11 (0.42-2.95)	0.830
Symptoms: (−)	5	26	26		
(+)	26	6	15	1.50 (0.33-6.65)	0.592
Dx procedure: Bronchoscopy	23	11	13		
Surgical biopsy	8	15	26	0.69 (0.23-2.04)	0.507
Respiratory failure: (−)	21	11	26		
(+)	10	5	10	2.18 (0.77-6.22)	0.142
Use of corticosteroid: (−)	16	16	80		
(+)	15	10	13	3.20 (1.09-9.38)	0.034
Serum KL-6 (U/ml): < 1960	16	13	26		
1960 ≦	15	5	15	1.52 (0.58-4.00)	0.389
Serum SP-D (ng/ml): < 147	15	10	26		
147 ≦	15	8	15	1.80 (0.62-5.22)	0.278
Serum SP-A (ng/ml): < 79	16	11	26		
79 ≦	14	5	15	2.79 (0.965-8.06)	0.058
%VC: 87 ≦	11	15	34		
< 87	11	8	15	3.27 (0.79-13.52)	0.101
FEV1%: 86 ≦	12	8	16		
< 86	10	13	34	0.52 (0.14-1.87)	0.322
%DLco: 44 ≦	9	21	34		
< 44	8	5	13	9.98 (1.03-96.11)	0.046

CI indicates confidence interval; OS, overall survival; DLco, diffusing capacity of the lung for carbon monoxide; Dx, diagnosis; FEV, forced expiratory volume; HR, hazard ratio; KL-6, krebs von den lungen-6; MDS, myelodysplastic syndrome; SP-A, surfactant protein -A; sPAP, secondary pulmonary alveolar proteinosis; SP-D, surfactant protein -D; VC, vital capacity.

corticosteroids was associated with poor survival (p = 0.024) (Figure 3A). However, the number of patients treated with steroid therapy did not differ between mild and severe MDS groups. A %DLco of <44% (Figure 3B) predicted poor prognosis (p = 0.019), whereas %vital capacity (%VC), forced expiratory volume (FEV) 1.0%, serum KL-6, SP-D, and surfactant protein-A (SP-A) did not.

Discussion

For the first time, we evaluated the prognosis of MDS/sPAP in a substantial cohort of patients, comparing mild and severe MDS as classified according to WPSS criteria. Our data demonstrate that the duration from diagnosis of MDS to diagnosis of sPAP was longer in mild MDS than that in severe MDS, but the survival probability was similar after the diagnosis of sPAP regardless of MDS severity. As a whole, occurrence of PAP appeared to worsen the prognosis of patients with mild MDS. This result is supported by the fact that the major cause of death was not MDS-associated but rather sPAP-associated respiratory failure or infections. Prior to our report, 21 cases with MDS/sPAP have been reported

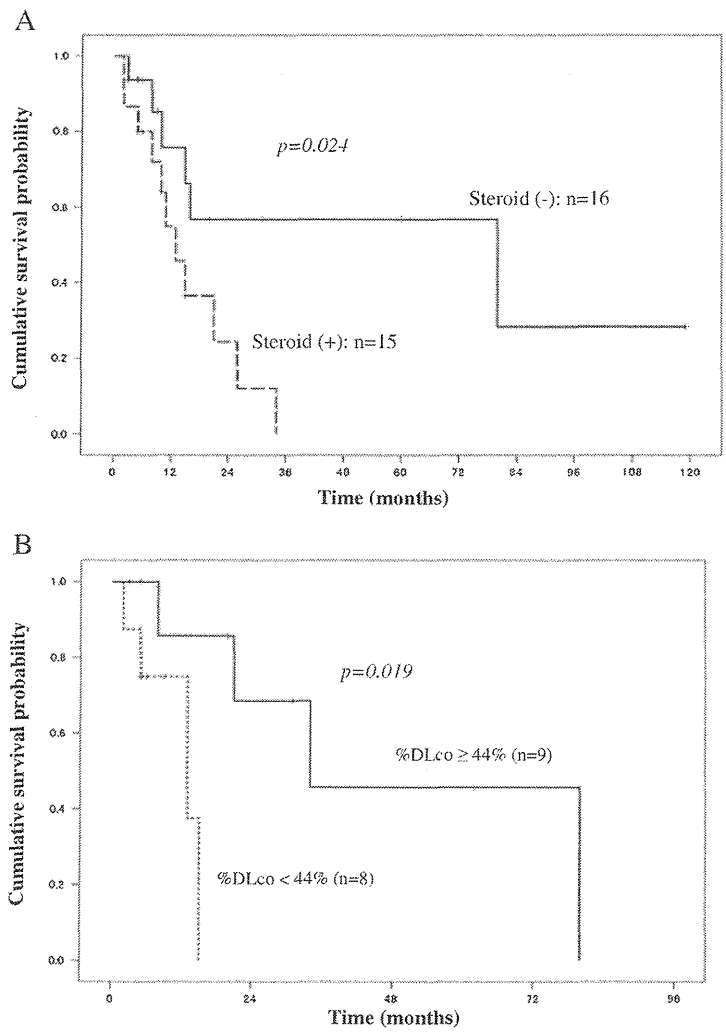

Figure 3 Risk factors for the prognosis of secondary PAP in patients with MDS. A: Cumulative survival probability after diagnosis of secondary PAP (sPAP) in myelodysplastic syndrome (MDS) cases with steroid therapy (n = 15) and in MDS cases without steroid therapy (n = 16). The horizontal axis indicates the month after the diagnosis of sPAP, and the vertical axis indicates the cumulative survival probability. The number of cases that received steroid therapy in the mild and severe MDS groups were six (46%) and nine (59%), respectively. **B**: Cumulative survival probability after diagnosis of secondary PAP (sPAP) in myelodysplastic syndrome (MDS) cases with diffusing capacity of the lung for carbon monoxide (%DLco) of <44% (n = 8) and in MDS cases with%DLco of >44% (n = 9). The horizontal axis indicates month after the diagnosis of sPAP, and the vertical axis indicates the cumulative survival probability.

[8-18]. Most reports [9-18,21-24] describe a single case, whereas only two publications report multiple cases [8,25]. The clinical course described in these reports suggest a poor prognosis for MDS/sPAP patients, but no prior report clearly quantifies the outlook for these patients and compares this to a predicted outcome by using validated prognostic scores such as WPSS. MDS/sPAP is so rare a disease that neither pulmonologists nor hematologists encounter such patients very often. In fact, in more than 10 years, 2 to 5 cases were diagnosed as MDS/sPAP annually in our analyses for serum GM-CSF autoantibody in over 600 diagnosed PAP cases from all over Japan; thus, we have finally reached cumulative 31 cases with MDS/sPAP.

According to the WHO criteria, 20 of the 31 cases were RA/RARS, 5 cases were RCMD, and 6 cases were RAEB1-2. The proportion of the number for each subtype in the total number of cases was comparable to literature data in terms of frequency and subtype distribution, suggesting that the risk of PAP complicating MDS is similar regardless of the subtype of MDS. It is speculated that AMs in patients with MDS derive from abnormal bone marrow precursor cells and are defective in both surfactant homeostasis and host defense, hence the progression of PAP and PAP-associated infections even in cases with mild MDS. Previous studies reported that in the absence of complicating sPAP, the five-year survival probability for patients with RA and RARS was 74% [26], whereas our cases with RA plus RARS had substantially inferior prognosis (0.69) (Additional file 2: Figure S2).

It is noteworthy that treatment with corticosteroids was associated with a markedly inferior prognosis. In Japan, steroid therapy often has been used for PAP but no evidence of its efficacy has been found. To our surprise, 15 of 31 cases had undergone steroid therapy during the course of sPAP. Our data reveal that steroid-treated patients had worse prognosis than did patients without steroid therapy. Given that the predominant cause of death was infective complications potentially exacerbated by steroid-related immunosuppression, these data clearly caution against the use of steroid therapy in such patients.

Treatment of MDS/sPAP should be directed toward the underlying malignancy, i.e., MDS. It should also aim at restoring hematopoietic function, either through allogeneic bone marrow transplant, which has curative potential for both MDS and sPAP, or through hypomethylating agent therapy for MDS, which can restore numerical hematologic parameters. However, functional cellular defects will likely remain, as such therapy does not necessarily eradicate the underlying clone, but rather enhances cellular differentiation [27,28]. Nevertheless, seven cases with MDS/sPAP to date in our cohort had undergone transplantation therapy. Of those, three patients died of pneumonia within three months of the transplantation therapy. Therefore, we do not have any convincing evidence to recommend transplantation therapy in the early stages of the disease. Although whole-lung lavage and segmental bronchial lavage were performed in 10 patients, only 3 cases showed the efficacy of lung-lavage therapy.

Infection often coexists with MDS/sPAP, although the causal relationship between PAP and infection is not clear. Superimposed infection accounts for a significant degree of morbidity and mortality in patients with sPAP. In the present study, 11 among 17 cases with fatal outcomes developed fatal infectious diseases. Considering that this complication was observed in the mild MDS and severe MDS groups, pneumonia accompanied with sPAP might be the trigger of fatal infection. Nevertheless, the present number of cases (31) is too small for accurate prognosis evaluation of MDS/sPAP; future international collaboration may be necessary to overcome this difficulty.

Conclusions

Complication of sPAP is an important risk factor in the prognosis of MDS. We believe that the present data will contribute to the management and treatment of the disease.

Additional files

Additional file 1: Figure S1. Survival curves after diagnosis of MDS in each mild and severe MDS.

Additional file 2: Figure S2. Survival curves in each MDS groups classified by WHO-criteria.

Abbreviations

AM: Alveolar macrophage; AML: Acute myeloid leukemia; ANC: Absolute neutrophil count; BALF: Bronchoalveolar lavage fluid; CEA: Carcinoembryonic antigen; CT: Computed tomography; DLco: Diffusing capacity of the lung for carbon monoxide; Dx: Diagnosis; FEV: Forced expiratory volume; GM-CSF: Granulocyte macrophage colony-stimulating factor; HbG: Hemoglobin; HRCT: High-resolution computed tomography; KL-6: Krebs von den Lungen-6; MDS: Myelodysplastic syndrome; PAP: Pulmonary alveolar proteinosis; PLT: Platelets; RA: Refractory anemia; RARS: Refractory anemia with ringed sideroblasts; RAEB: Refractory anemia with blasts; RBC: Red blood cell; RCMD: Refractory anemia with multilineage dysplasia; SD: Standard deviation; SP-A: Surfactant protein-A; sPAP: Secondary pulmonary alveolar proteinosis; SP-D: Surfactant protein-D; VC: Vital capacity; WHO: World Health Organization; WPSS: WHO classification-based prognostic scoring system.

Competing interests

Financial/non-financial competing interests

The authors report no potential conflicts of interest with any companies or organizations whose products or services are mentioned in this article.

Authors' contributions

The authors take responsibility and vouch for the completeness and accuracy of the data and analyses. HI and KN are the guarantors of the entire manuscript. HI and JS contributed to the study concept and design, coordination of data acquisition, and writing of the article. RT, YI, NU, AN, YK,

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References

1. Rosen SH, Castleman B, Liebow AA: **Pulmonary alveolar proteinosis.** *N Engl J Med* 1958, **258**:123–1143.
2. Seymour JF, Presneill JJ: **Pulomary alveolar proteinosis: progress in the first 44 years.** *Am J Respir Crit Care Med* 2002, **166**:215–235.
3. Kitamura T, Tanaka N, Watanabe J, Uchida K, Kanegasaki S, Yamada Y, Nakata K: **Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte-macrophage colony stimulating factor.** *J Exp Med* 1999, **190**:875–880.
4. Ishii H, Tazawa R, Kaneko C, Saraya T, Inoue Y, Hamano E, Kogure Y, Tomii K, Terada M, Takada T, Hojo M, Nishida A, Ichiwata T, Trapnell BC, Goto H, Nakata K: **Clinical features of secondary pulmonary alveolar proteinosis: pre-mortem cases in Japan.** *Eur Respir J* 2011, **37**:465–468.
5. Cazzola M, Malcovati L: **Myelodysplastic syndrome: coping with ineffective hematopoiesis.** *N Engl J Med* 2005, **352**:536–538.
6. Vardiman JW, Harris NL, Brunning RD: **The World Health Organization (WHO) classification of the myeloid neoplasms.** *Blood* 2002, **100**:2292–2302.
7. Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, Giagounidis A, Hildebrandt B, Bernasconi P, Knipp S, Strupp C, Lazzarino M, Aul C, Cazzola M: **Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndrome.** *J Clin Oncol* 2007, **25**:3503–3510.
8. Nishida A, Miyamoto A, Yamamoto H, Uchida N, Izutsu K, Wake A, Ohta Y, Fujii T, Araoka H, Taniguchi S, Kishi K: **Possible association of trisomy 8 with secondary pulmonary alveolar proteinosis in myelodysplastic syndrome.** *Am J Respir Crit Care Med* 2011, **184**:279–280.
9. Asai Y, Ouchi H, Ohosima T, Nakano R, Yamano Y, Inoshima I, Yamauchi T, Fukuyama S, Inoue H, Nakanishi Y: **A case of secondary pulmonary alveolar proteinosis associated with myelodysplastic syndrome, complicated with disseminated *M. abscessus* infection.** *Nihon Kokyuki Gakkai Zasshi* 2009, **47**:1120–1125.
10. Tabata S, Shimoji S, Murase K, Takiuchi Y, Inoue D, Kimura T, Nagai Y, Mori M, Togami K, Kurata M, Ito K, Hashimoto H, Matushita A, Nagai K, Takahashi T: **Successful allogeneic bone marrow transplantation for myelodysplastic syndrome complicated by severe pulmonary alveolar proteinosis.** *Int J Haemol* 2009, **90**:407–412.
11. Fukuno K, Tomonari A, Tsukada N, Takahashi S, Ooi J, Konuma T, Uchiyama M, Fujii T, Endo T, Iwamoto A, Oyaizu N, Nakata K, Moriwaki H, Tojo A, Asano S: **Successful cord blood transplantation for myelodysplastic syndrome resulting in resolution of pulmonary alveolar proteinosis.** *Bone Marrow Transplant* 2006, **38**:581–582.
12. Ohnishi T, Yamada S, Shijubo N, Takagi-Takahashi Y, Itoh T, Takahashi H, Satoh M, Koba H, Nakata K, Abe S: **Secondary pulmonary alveolar proteinosis associated with myelodysplastic syndrome.** *Intern Med* 2003, **42**:187–190.
13. Yokomura K, Chida K, Suda T, Miwa S, Nakano H, Kuwata H, Suzuki K, Matsuda H, Asada K, Nakamura Y, Inui N, Shirai M, Suzuki K, Nakamura H: **Secondary pulmonary alveolar proteinosis associated with myelodysplastic syndrome.** *Nihon Kokyuki Gakkai Zasshi* 2002, **40**:599–604.
14. Yoshioka Y, Ohwada A, Harada N, Satoh N, Sakuraba S, Dambara T, Fukuchi Y: **Increased circulating CD16+ CD14dim monocytes in a patient with pulmonary alveolar proteinosis.** *Respirology* 2002, **7**:273–279.
15. Ito T, Yoshii C, Imanaga T, Hayashi T, Kawanami K, Kido M: **A case of pulmonary alveolar proteinosis complicated with pneumoconiosis and myelodysplastic syndrome.** *Nihon Kokyuki Gakkai Zasshi* 2001, **39**:710–715.
16. Kajiume T, Yoshimi S, Nagita A, Kobayashi K, Kataoka N, Nakajima M, Matsushima T: **A case of myelodysplastic syndrome complicated by pulmonary alveolar proteinosis with a high serum KL-6 level.** *Pediatr Hematol Oncol* 1999, **16**:367–371.
17. Terashima T, Nakamura H, Meguro S, Fujimori H, Yamaguchi K, Kanazawa M, Kato R, Kobayashi K: **Pulmonary alveolar proteinosis associated with myelodysplastic syndrome.** *Nihon Kokyuki Gakkai Zasshi* 1995, **33**:645–651.
18. Nakata K, Tamura T, Aikawa K, Yoshida H, Mikuni O, Inoue M, Nasuhara K: **A case of pulmonary alveolar proteinosis complicated with myelodysplastic syndrome.** *Nihon Naki Gakkai Zasshi* 1991, **80**:106–107.
19. Standing Committee on Human Cytogenetics Nomenclature: **ISCN: an international system for human cytogenetic nomenclature-report of the standing committee on human cytogenetic nomenclature.** *Birth Defects Orig Ser* 1985, **21**:1–117.
20. Greenberg P, Cox C, Lebeau MM, Fenaux P, Morel P, Sanz M, Vallespi T, Hamblin T, Oscier D, Ohyashiki K, Toyama K, Aul C, Mufti G, Bennett J: **International scoring system for evaluating prognosis in myelodysplastic syndromes.** *Blood* 1997, **8**:2079–2088.
21. Xue Y, Han Y, Li T, Chen S, Zhang J, Pan J, Wu Y, Wang Y, Shen J: **Pulmonary alveolar proteinosis as a terminal complication in a case of myelodysplastic syndrome with idic(20q-).** *Acta Haematol* 2010, **123**:55–58.
22. Chung JH, Pipavath SJ, Myerson DH, Godwin D: **Secondary pulmonary alveolar proteinosis: a confusing and potentially serious complication of hematologic malignancy.** *J Thorac Imaging* 2009, **24**:115–118.
23. Xie LX, Zhao TM, Wang QY, Chen LA, Li AM, Wang DJ, Qi F, Liu YN: **Secondary pulmonary alveolar proteinosis associated with myelodysplastic syndrome.** *Chin Med J* 2007, **120**:1114–1116.
24. Pollack SM, Gutierrez G, Ascensao J: **Pulmonary alveolar proteinosis with myeloprolifereative syndrome with myelodysplasia: bronchoalveolar lavage reduces white blood cell count.** *Am J Hematol* 2006, **81**:634–636.
25. Shoji N, Ito Y, Kimura Y, Nishimaki J, Kuriyama Y, Tauchi T, Yamaguchi M, Payzulla D, Ebihara Y, Ohyashiki K: **Pulmonary alveolar proteinosis as a terminal complication in myelodysplastic syndrome: a report of four cases detected on autopsy.** *Leuk Res* 2002, **26**:591–595.
26. Malcovati L, Porta GD, Pascutto C, Invernizzi R, Boni M, Travaglino E, Passamonti F, Arcaini L, Maffioli M, Bermasconi P, Lazzarino M, Cazzola M: **Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making.** *J Clin Oncol* 2005, **23**:7594–7603.
27. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, Schoch R, Gattermann N, Sanz G, List A, Gore SD, Symour JF, Bennett JM,

Byrd J, Backstrom J, Zimmerman L, Mckenzie D, Beach C, Silverman LR: Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomized, open-label, phase III study. *Lancet Oncol* 2009, 10(3):223–232.

28. Gangatharan SA, Carney DA, Campbell LJ, Prince HM, Keneally MK, Seymour JF: Cytogenetic response is not a prerequisite for clinical response in patients with myelodysplastic syndromes treated with azacitidine. *Eur J Haematol* 2011, 87(2):186–188.

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REVIEW ARTICLE
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Novel aspects on the pathogenesis of *Mycoplasma pneumoniae* pneumonia and therapeutic implications

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Mycoplasma pneumoniae (Mp) is a leading cause of community acquired pneumonia. Knowledge regarding Mp pneumonia obtained from animal models or human subjects has been discussed in many different reports. Accumulated expertise concerning this critical issue has been hard to apply clinically, and potential problems may remain undiscovered. Therefore, our multidisciplinary team extensively reviewed the literature regarding Mp pneumonia, and compared findings from animal models with those from human subjects. In human beings, the characteristic pathological features of Mp pneumonia have been reported as alveolar infiltration with neutrophils and lymphocytes and lymphocyte/plasma cell infiltrates in the peri-bronchovascular area. Herein, we demonstrated the novel aspects of Mp pneumonia that the severity of the Mp pneumonia seemed to depend on the host innate immunity to the Mp, which might be accelerated by antecedent Mp exposure (re-exposure or latent respiratory infection) through up-regulation of Toll-like receptor 2 expression on bronchial epithelial cells and alveolar macrophages. The macrolides therapy might be beneficial for the patients with macrolide-resistant Mp pneumonia via not bacteriological but immunomodulative effects. This exhaustive review focuses on pathogenesis and extends to some therapeutic implications such as clarithromycin, and discusses the various diverse aspects of Mp pneumonia. It is our hope that this might lead to new insights into this common respiratory disease.

Keywords: *Mycoplasma pneumoniae* pneumonia, animal models, epidemiology, pathology, pathogenesis

INTRODUCTION

Mycoplasma pneumoniae (Mp) was first isolated in tissue culture from the sputum of a patient with primary atypical pneumonia by Eaton et al. (1944). This "Eaton's agent" was shown to be a *Mycoplasma* species in 1961. Chanock et al. succeeded in culturing Eaton's agent in mammalian cell-free medium and proposed the taxonomic designation Mp in 1963 (Chanock et al., 1962; Chanock, 1963). Mp is a unique organism that lacks a cell wall in any circumstances, and does not need a host cell for replication. This organism causes a variety of clinical presentations, from self-limiting to life-threatening. The disease severity seems to depend on the degree of host's defenses. In this review, we focused on the pathogenesis of Mp pneumonia from the perspective of host defenses, based on findings from our mouse models.

EPIDEMIOLOGY

Mp is one of the most common pathogens of community-acquired pneumonia (CAP) in adults (**Table 1**). In general, both regional differences and varying periods of surveillance may influence the results of etiological studies of infectious diseases. **Table 1** summarizes the proportions of adult Mp pneumonia among CAP populations enrolled in several large-scale studies conducted in various countries (Marston et al., 1997; Ngeow et al., 2005; Arnold et al., 2007; Von Baum et al., 2009; Cilloniz et al., 2011). Mp pneumonia accounted for 10.6–17.0 and 3.0–20.8% of CAP in out- or in-patients settings, respectively, and the frequency of ICU admission was relatively low (2–3.6%). Arnold et al. showed that Mp is the most common atypical pneumonia pathogen, accounting for 11–15% of CAP throughout the world (Arnold et al., 2007). Serological studies in Denmark over a 50-year period showed that Mp infections exhibit epidemic periodicity every 3–5 years, but this trend now seems to be getting obscured (Lind et al., 1997). Mp pneumonia occurs at any age, but the incidence is less common in elderly, as compared with young, adults (Lim et al., 2009), and is highest among school-aged children (Foy et al., 1979).

Macrolides were recommended for treatment of microbiologically defined Mp pneumonia. However, macrolide-resistant