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Incidence of myeloperoxidase anti-neutrophil cytoplasmic antibody positivity and microscopic polyangitis in the course of idiopathic pulmonary fibrosis

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KEYWORDS
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Summary
Background: Pulmonary fibrosis is a manifestation of microscopic polyangitis (MPA), and often precedes the detection of MPA. The prevalence and sequence of myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA) and MPA in patients initially diagnosed with idiopathic pulmonary fibrosis (IPF) have not been precisely elucidated.
Methods: We enrolled 61 consecutive patients with IPF and measured the MPO-ANCA titers at initial presentation and during the follow-up period. Clinical, radiologic and histologic features of MPO-ANCA-positive cases were examined.
Results: Of 61 patients, 3 (4.9%) had positive MPO-ANCA titers at the initial presentation of IPF. During the disease course, MPO-ANCA-positive conversion occurred in 6 patients and the prevalence of ANCA increased to 14.8%. Among the nine patients positive for MPO-ANCA, two patients developed MPA during follow-up. Histologic features of MPO-ANCA-positive pulmonary fibrosis were compatible with the usual interstitial pneumonia pattern in which alveolar hemorrhage and capillaritis were not observed. The patients with MPO-ANCA-positive conversion showed increased percentages of bronchoalveolar lavage eosinophils and more frequent

Abbreviations: ANCA, anti-neutrophil cytoplasmic antibodies; BAL, bronchoalveolar lavage; CVD-IP, collagen vascular disease-associated interstitial pneumonia; HRCT, high-resolution computed tomography; IPF, idiopathic pulmonary fibrosis; MPA, microscopic polyangitis; MPO, myeloid enzymes myeloperoxidase; NSIP, nonspecific interstitial pneumonia.

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complication of pulmonary emphysema compared to those with MPO-ANCA-negative IPF.
Conclusions: The findings of the present study demonstrated that patients with an initial diagnosis of IPF occasionally acquire MPO-ANCA, which develops to MPA during the disease course of IPF. The presence of pulmonary eosinophilia and low attenuation areas on computed tomography scans might be predictive of MPO-ANCA positive conversion.

Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA) are directed against enzymes in the granules of polymorphonuclear leukocytes and the major ANCA antigens are the myeloid enzymes myeloperoxidase (MPO) and proteinase 3.[1,2] ANCA plays a role in both neutrophil activation and direct tissue injury in vitro,[3] suggesting that ANCA is involved in the pathogenesis of systemic vascular disorders. Clinically, ANCA is an important serum biomarker for diagnosing and monitoring systemic vasculitis.[4,5]

Pulmonary lesions often accompany microscopic polyangitis (MPA) and manifest in patients with MPO-ANCA. Pulmonary fibrosis was present in 36% of patients with MPA at the time of diagnosis.[6] A previous report demonstrated that chronic interstitial lesions are common histologic features in patients with MPO-ANCA based on the pathologic reviews.[7,8] Thus, pulmonary fibrosis is now a recognized manifestation of MPA. Nada et al. reported three patients initially diagnosed with idiopathic pulmonary fibrosis (IPF) that developed pulmonary-renal vasculitis.[9] Since then, similar cases have accumulated in small clinical series, suggesting that patients with IPF can develop MPA. However, the association and sequence of pulmonary fibrosis and MPO-ANCA positivity and vasculitis has not been elucidated.

Here, we examined the MPO-ANCA positivity in consecutive patients with an initial diagnosis of IPF at hospital presentation and during the follow-up period, as well as the incidence of the development to MPA. In addition, we examined clinical, radiologic and histologic features of MPO-ANCA-positive patients. We also aimed to characterize the clinical picture predictive of MPO-ANCA-positive conversion in IPF.

Methods

Patient selection

This study was approved by the Institutional Ethics Committee approval of Oita University (No.151). We reviewed 85 consecutive patients with IPF diagnosed at the Oita University Hospital during the period between January 2000 and December 2009. The diagnosis of IPF was made on the basis of the criteria of the American Thoracic Society/European Respiratory Society international multidisciplinary consensus classification.[10] Patients were considered to have undifferentiated connective tissue disease (UCTD) if their medical record identified signs/symptoms and laboratory findings that met the criteria for UCTD as previously published.[11]

We analyzed the incidence of the development to MPA during follow-up of the patients. MPA was diagnosed based on the Chapel Hill Consensus Conference nomenclature of systemic vasculitis.[12] To elucidate the incidence of MPO-ANCA positivity and MPO-ANCA-positive conversion in patients with IPF diagnosed at initial presentation, we excluded the patients with critical diseases, such as systemic vasculitis, malignancies and acute exacerbation of pulmonary fibrosis. One patient manifesting MPA at the first medical examination was excluded from this study. Also, 17 patients with malignancies, one patient with sepsis due to severe pneumonia and five patients who were complicated by acute exacerbation of the disease at initial presentation were also excluded.

Pulmonary function testing and bronchoalveolar lavage procedure

Pulmonary function testing was performed on all 61 patients at initial presentation. The carbon monoxide diffusing capacity was available in 51 patients.

Bronchoalveolar lavage (BAL) was performed on the characterization of the inflammatory cellular population. Briefly, a fiberoptic bronchoscope was wedged into the middle lobe or lingula. Saline solution was instilled in 3 aliquots of 50 ml and then BAL fluid specimens from the subjects were collected. After the total cell concentration was calculated, the cells were cytospun at 400 rpm for 5 min and 300 cells were differentially counted.

Chest high-resolution computed tomography scanning and histologic examination

All patients with IPF underwent chest high-resolution computed tomography (HRCT) scanning at the time of initial presentation. The chest HRCT scans were obtained with a window setting for lung parenchyma (window width, 1600—1800 HU; window level, −600 to −700 HU) and mediastinum (window width, 300—350 HU; window level, 25—40 HU).

A surgical lung biopsy was available in 26 patients. In the MPO-ANCA positive IPF cases, a surgical biopsy was available in 6 and postmortem examination in 2 patients. In three of these patients, the biopsy was performed before MPO-ANCA became positive. The kidney biopsies were available in six patients with MPO-ANCA positivity. Five had needle biopsies and one an autopsy specimen. Serum MPO-ANCA was positive at the time of kidney biopsy.

Measurement of MPO-ANCA

We measured MPO-ANCA in 61 IPF patients at the initial hospital presentation and during the follow-up period. Blood samples were obtained from clotted blood following

centrifugation at 1500 × *g* at 4 °C for 10 min, and stored at −80 °C until the measurements were performed. MPO-ANCA was measured using an enzyme-linked immunosorbent assay kit (NIPRO, Japan) and the values above 20 EU were considered to be positive.

Statistics

Statistical analyses were performed using SPSS II software (SPSS Inc, Chicago, IL). Data are expressed as median and range. The Mann−Whitney *U*-test was applied to elucidate the difference between the two independent groups. Fisher's exact probability test was used to compare dichotomous variables between the two groups. Kaplan−Meier analysis was used to compare survival between groups. A p value of less than 0.05 was considered significant.

Results

Patient characteristics

The characteristics and laboratory findings of the study population at initial hospital presentation are shown in Table 1. We enrolled 61 patients including 46 men and 15 women. The median age was 68 years (range 40−82 years). Forty-five patients were smokers.

The prevalence of MPO-ANCA and MPA in patients with IPF

In three of 61 patients (4.9%), MPO-ANCA was positive at the time of diagnosis (Table 1). All 61 patients were followed-up in our hospital and chronologic measurement of MPO-ANCA was performed until December 2010. During the follow-up period (median 40 months; range, 1−121 months), six patients exhibited positive conversion of MPO-ANCA. In contrast, negative conversion was not observed in the MPO-ANCA-positive patients during the course of observation without treatment. The total prevalence of MPO-ANCA positivity in the 61 subjects was 14.8%.

Table 1 Characteristics of study population at initial hospital presentation.

Characteristics	
Number of patients	61
Age, yr	68 (40−82)
Male/female	46/15
Smoking status	
Never-smoker	16
Smoker	45
Laboratory data	
WBC	6800 (3400−15,100)
CRP	0.2 (0.01−9.83)
LDH	277 (90−710)
Cre	0.77 (0.19−1.64)
ANA positive	31 (50.8%)
MPO-ANCA positive	3 (4.9%)

Abbreviations: IPF: idiopathic pulmonary fibrosis; WBC: white blood counts; CRP: C reactive protein; LDH: lactate dehydrogenase; Cre: creatinine; ANA: anti-nuclear antibody; ANCA: anti-neutrophil cytoplasmic antibody; MPO: myeloperoxidase.

Clinical characteristics of MPO-ANCA positive pulmonary fibrosis

To clarify the characteristics of the MPO-ANCA-positive pulmonary fibrosis, we evaluated the clinical, radiologic and histologic characteristics of the nine patients positive for MPO-ANCA (Table 2). Median age was 69 years (range, 57−75 years). All patients were men and smokers (2 current smokers and 7 ex-smokers). Three patients were positive for MPO-ANCA initially and 6 became positive during the follow-up period. The median duration between initial IPF diagnosis and conversion to MPO-ANCA positivity was 23 months (range. 0−71 months). The maximum levels of MPO-ANCA titers ranged from 22 to 1460 EU. At the time of detection, 3 patients presented with prolonged or recurrent fever, 2 patients presented with myalgia and a patient demonstrated unintentional weight loss. Lung histopathology was examined in 7 MPO-ANCA positive cases. A surgical lung biopsy was performed for 6 patients and 3 of these were examined prior to conversion. The histologic pattern was consistent with that of usual interstitial pneumonia. Alveolar hemorrhage or small vessel vasculitis was not observed. Prominent lymphoid follicle proliferation was observed in two cases. Postmortem examination of the lung for two cases showed alveolar hemorrhage, but no evidence of small vessel vasculitis. We also performed histopathologic examination of the kidney by a needle biopsy or at postmortem examination. We confirmed a diagnosis of MPA in 2 cases based on findings of crescentic glomerulonephritis compatible with MPO-ANCA-related nephropathy. The histopathological findings of the remaining 4 cases were mesangial proliferative glomerulonephritis, chronic tubulointerstitial nephritis, minor glomerular abnormalities, and normal findings, respectively. Thus, they were not diagnosed as MPO-ANCA related nephropathy. Three out of 9 patients (33%) with positive MPO-ANCA met UCTD criteria, whereas only 2 out of 52 (3.8%) patients with negative MPO-ANCA met the UCTD criteria. The prevalence of UCTD in MPO-ANCA-positive cases was statistically higher than that in MPO-ANCA-negative cases. ($p = 0.02$).

Treatment and outcome

We retrospectively analyzed whether the treatment of the MPO-ANCA-positive patients was different to that given to IPF patients. As shown in Table 2, eight of nine (89%) patients with positive MPO-ANCA were treated with corticosteroids, whereas 26 of 52 (50%) patients with IPF were treated with corticosteroids. There was a statistically significant difference ($p = 0.036$). Immunosuppressants including azathioprine, cyclophosphamide or mizorbine were administered for four (44%) patients with MPO-ANCA-positive fibrosis and only one (2%) patients with IPF. Pirfenidone was given to seven patients with IPF. Thus treatment was different between MPO-ANCA-positive fibrosis and IPF. As for MPO-ANCA-positive fibrosis, the treatment was started for progressive

Table 2 Clinical and pathologic features of MPO-ANCA-positive pulmonary fibrosis.

Case	Age	Sex	Smoking status	MPO-ANCA (EU)	Autoantibodies	[a]Duration between the diagnosis and MPO-ANCA detection	Seroconversion of MPO-ANCA titers	Additional symptoms at the time of MPO-ANCA positive	Histopathology of lung	Vasculitis	Diagnosis	Treatment	Outcome
1	70	M	Ex	120	ANA (1:80), Jo-1(+)	53	Yes	Fever, myalgia	N.D.	Kidney	MPA	PSL, AZP	Died (acute exacerbation)
2	57	M	Ex	171	ANA (1:80) SS-A(+)	11	Yes	None	UIP pattern	N.D.	IPF	PSL, CPA	Died (CMV infection)
3	69	M	Cur	133	ANA (1:40)	69	Yes	None	UIP pattern	N.D.	IPF	PSL	Died (acute exacerbation)
4	70	M	Ex	1460	ANA (1:80)	23	Yes	Fever	UIP pattern	Kidney	MPA	PSL, MZR	Died (pneumothorax)
5	75	M	Ex	576	RA (2+) dsDNA(+)	0	N.D.	Unintentional weight loss	UIP pattern	N.D.	UCTD	PSL	Died (acute exacerbation)
6	71	M	Ex	22	RF 669 IU/ml	0	N.D.	Fever	UIP pattern	N.D.	UCTD	PSL	Survived
7	67	M	Cur	31	ANA (1:40) RF 90 IU/ml	0	N.D.	Myalgia	UIP pattern	N.D.	UCTD	PSL, AZP	Died (acute exacerbation)
8	58	M	Ex	1400	N.D.	71	Yes	None	UIP pattern	N.D.	IPF	PSL	Survived
9	67	M	Ex	24	ANA (1:40)	44	Yes	None	N.D.	N.D.	IPF	None	Survived

Abbreviations: Ex: Ex-smoker; Cur: current smoker; N.D.: not detected; UIP: usual interstitial pneumonia; MPA: microscopic polyangitis; UCTD: undifferentiated connective tissue disease; IPF: idiopathic pulmonary fibrosis; PSL: prednisolone; AZP: azathioprine; CPA: cyclophosphamide; MZR: mizorbine; None: non-treatment.

[a] Duration between the diagnosis of IPF and MPO-ANCA detection (months).

respiratory failure in four patients, for systemic vasculitis with definite MPA diagnosis in two, for persistent fever in one, and refractive myalgia in one. Six patients died during the follow-up period. Four patients died of acute exacerbation of pulmonary fibrosis, one died of intractable pneumothorax, and one died of cytomegalovirus infection. As for IPF, therapy was started for progressive respiratory failure in all patients. Eight patients died of acute exacerbation of pulmonary fibrosis, 6 of progressive respiratory failure, 6 of pneumonia and 3 of lung cancer. The median survival of patients with MPO-ANCA-positive fibrosis and IPF was 62 months (95% CI 29.13—94.87) and 63 months (95% CI 44.55—81.42), respectively. There was no significant difference between the two groups ($p = 0.9266$).

High-resolution computed tomography

In the initial HRCT scans of the nine MPO-ANCA-positive patients, there were subpleural reticular opacities, traction bronchiectasis and honeycombing (Fig. 1). These findings were also frequently observed in MPO-ANCA-negative cases. No differences were found between the two groups.

Characteristics of cases developing MPO-ANCA positive conversion

In an attempt to predict which cases would develop MPO-ANCA positivity, we compared data between MPO-ANCA-positive and -negative PF patients (Table 3). At the time of first medical examination, the positive anti-nuclear antibodies were found in 65% of the patients with MPO-ANCA-positive fibrosis and 50% of IPF. The incidence of positive anti-nuclear antibodies was not different between the two groups ($p = 0.488$). Among several autoantibodies, the positive rate of rheumatoid factor or RA test of MPO-ANCA-positive fibrosis was significantly higher than that of IPF ($p = 0.006$). As for pulmonary function test, value of carbon monoxide diffusion capacity (DLCO) of the MPO-ANCA-positive cases was lower than that of the negative group. The percentage of BALF eosinophils in the MPO-ANCA-positive group was higher than that in the negative group ($p = 0.04$). Low attenuation areas on HRCT were observed more frequently in the MPO-ANCA-positive group as compared with the MPO-ANCA-negative group ($p = 0.02$).

Discussion

This report is the first to describe the prevalence and chronology of MPO-ANCA positivity and MPA in consecutive patients with an initial diagnosis of IPF. In the present study, 3 of 61 patients exhibited positive MPO-ANCA titers when first evaluated. During follow-up six addition patients became positive for MPO-ANCA. Thus, the prevalence of MPO-ANCA positivity increased over time. Among the nine patients with positive MPO-ANCA titers, the development of systemic vasculitis was confirmed in two.

In the present study, we measured MPO-ANCA for consecutive patients with an initial diagnosis of IPF and the prevalence of MPO-ANCA at the initial presentation was 4.9% and increased to 14.8% during the follow-up period. Previously, Nozu and colleagues reported higher prevalence of MPO-ANCA positivity. They showed that 19 patients (35.8%) were ANCA-positive (17 patients with positive MPO-ANCA and 2 patients with positive proteinase 3-ANCA). However there may be a selection bias in their study because ANCA was measured based only on the referring doctor's decision resulting in relatively high percentage of female patients.[13]

Previously reported series indicated that IPF precedes the development of vasculitis in the majority cases.[9,14—17] The precise mechanism of MPO-ANCA production in IPF, however, remains unclear. Given that MPO-ANCA is often detected in patients with suppurative respiratory disease

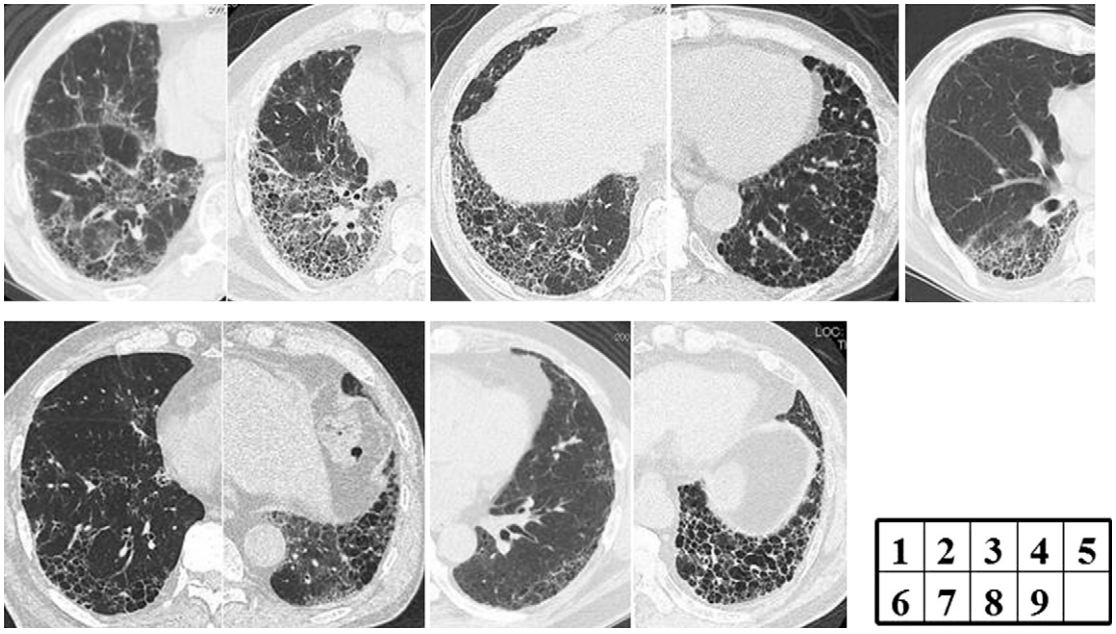

Figure 1 High-resolution computed tomography scans of nine MPO-ANCA-positive cases initially diagnosed with idiopathic pulmonary fibrosis (IPF). A; case 1; B, case 2; C, case 3; D, case 4, E, case 5; F, case 6; G, case 7; H, case 8; I, case 9.

Table 3 Comparisons between patients with pulmonary fibrosis developing MPO-ANCA-positive conversion and patients with MPO-ANCA-negative idiopathic pulmonary fibrosis.

Characteristics	MPO-ANCA negative	MPO-ANCA positive	*p* value
Number of patients	52	9	
Age, yr	69 (50—82)	69 (57—75)	0.541
Male/female	37/15	9/0	0.064
Smoking status			
Never-smoker	16 (31.0%)	0 (0%)	0.051
Smoker	36 (69.0%)	9 (100%)	
Cough	33 (63.5%)	3 (33.3%)	0.093
DOE	29 (56.9%)	7 (77.8%)	0.193
Fever	4 (7.7%)	1 (11.1%)	0.563
Laboratory findings			
WBC	6715 (3400—11,900)	8850 (5420—15,100)	0.055
CRP	0.19 (0.01—4.92)	2.16 (0.06—9.83)	0.084
LDH	270 (90—710)	227 (148—457)	0.903
Cre	0.77 (0.19—1.64)	0.88 (0.61—1.52)	0.590
ANA, positive	26/52 (50%)	6/9 (67%)	0.488
RA test or RF, positive	5/46 (11%)	4/6 (66%)	**0.006**
Anti-SSA, positive	1/48 (2%)	1/6 (17%)	0.212
Anti-SS-B, positive	1/48 (2%)	0/5 (0%)	1.000
Anti-dsDNA, positive	1/43 (2%)	1/7 (14%)	0.263
Anti-Sm, positive	1/47 (2%)	1/6 (17%)	0.216
Anti-Scl70, positive	6/47 (13%)	0/5 (0%)	1.000
Anti-RNP, positive	2/46 (4%)	0/6 (0%)	1.000
Anti-Jo1, positive	4/47 (9%)	1/6 (17%)	1.000
Anti-centromere, positive	2/19 (11%)	0/3 (0%)	
Pulmonary function test			
%VC	75.7 (39.8—119.1)	66.9 (54.1—89.2)	0.888
FEV1.0%	82.8 (69.3—112)	82.5 (75.6—89.4)	0.672
%DLco	63.9 (22.7—96.4) ($n = 44$)	41.6 (22.1—79.6) ($n = 7$)	**0.040**
BAL, fluid, %			
Number of patients	46	6	
Total cells concentration	2.33 (0.9—7.44)	2.5 (1.00—3.55)	0.927
Macrophage	88 (34.7—99.0)	72.8 (41.4—87.5)	**0.020**
Lymphocytes	6.3 (0.6—31.3)	7.25 (2.0—13.0)	0.669
Neutrophils	3.0 (0—57.0)	6.7 (1.5—54.0)	0.222
Eosinophils	1.3 (0—10.3)	5.4 (0.9—14.5)	**0.003**
HRCT findings			
Groud-glass opacities	52 (100%)	9 (100%)	—
Interlobular septal thickning	52 (100%)	9 (100%)	—
Traction bronchiectasis	46 (88.4%)	9 (100%)	0.283
Consolidation	11 (26.8%)	1 (11.1%)	0.484
Honeycombing	42 (80.8%)	9 (100%)	0.175
Low attenuation areas	18 (34.6%)	8 (88.8%)	**0.002**

Abbreviations: DOE: dyspnea on effort; WBC: white blood cell count; CRP: C-reactive protein; Cre: creatinine; ANA: anti-nuclear antibody; RF: rheumatoid factor; VC: vital capacity; FEV_1%: predicted forced expiratory volume in 1 s; %DLco; predicted percent value of diffuse capacity of carbon monoxide; BAL; bronchoalveolar lavage.
Bold value signifies Mann—Whitney *U*-test, Fisher's extract probability test, $p < 0.05$, statistically significant.

and diffuse panbronchiolitis,[18,19] it may be possible that MPO-ANCA would be produced as a result of neutrophil destruction during the chronic inflammation process.

HRCT subpleural reticular opacities, traction bronchiectasis and honeycombing were observed in all MPO-ANCA-positive subjects, however these findings were also found in MPO-ANCA-negative IPF patients (80.8%). Nozu and colleagues reported a similar result: honeycombing was observed in 73% patients with ANCA-positive pulmonary fibrosis and in 54.8% patients with ANCA-negative pulmonary fibrosis.[13] Corresponding to the CT findings, the lung histopathology of MPO-ANCA positive pulmonary fibrosis matched the pattern for usual interstitial pneumonia in an autopsy series of 11 cases, in which vasculitis was observed in 5 cases.[20] Our series add new data of the biopsy histopathology by examining 6 MPO-ANCA-positive patients before the development of MPA. Moreover, three patients were examined before MPO-ANCA positive conversion. As a result, the histopathologic pattern was compatible with the usual interstitial pneumonia pattern and no alveolar hemorrhage or small vessel vasculitis was

observed in the lung specimens in any of the cases. Thus, it is difficult to clearly discriminate the MPO-ANCA-positive and MPO-ANCA-negative cases among patients with IPF based on radiologic and histologic findings, but honeycombing which is the most characteristic finding of IPF with MPO-ANCA positivity would be a stretch of one's imagination. In contrast, after MPA occurs, various CT findings including ground-glass attenuation, consolidation and nodules are observed in ANCA-positive lung fibrosis.[21]

In this series, we reviewed nine IPF patients with MPO-ANCA at initial presentation or during follow-up. Interestingly, they were all smokers and frequently demonstrated low attenuation areas on their HRCT scans and also showed lower DLco in comparison with MPO-ANCA-negative cases. Similarly, Foulon et al. reported that cumulative cigarette smoking tended to be higher in ANCA-positive patients with pulmonary fibrosis than in ANCA-negative controls.[22] A previous report by Sessa et al. described a higher prevalence of smoking in patients with rapidly progressive glomerulonephritis due to ANCA associated small vessel vasculitis.[23] Cigarette smoke is likely associated with MPO expression on epithelial cells and induces neutrophilic infiltration into the lung tissue through proinflammatory cytokine production.[24] Abundant MPO in the lung tissue might contribute to the generation of MPO-ANCA.

In patients who acquired MPO-ANCA we sought to determine the factors which predict this conversion. These patients had BAL eosinophilia and a higher coincidence of low attenuation areas on HRCT. We recommend measurement of MPO-ANCA during follow-up periods if these features were present initially.

MPA is a serious complication in MPO-ANCA-positive patients. Foulon and colleagues reported that MPA occurred in 5 of 6 (83.3%) patients with MPO-ANCA positivity and pulmonary fibrosis.[22] In our study, MPA was histologically diagnosed in 2 of 9 (22.2%) patients with positive MPO-ANCA, which may indicate a lower prevalence of MPA. The difference in the prevalence may be due to a recall bias with identification of the ANCA-positive patients with vasculitis in the previous study.[11]

Rheumatologic studies have estimated that as many as 25% of patients with features of a systemic autoimmune disease do not fulfill American College of Rheumatology (ACR) classification criteria for connective tissue disease (CTD). These patients are considered to have diffuse or undifferentiated connective tissue disease (UCTD).[25–29] Kinder et al. recently showed that the majority of patients (83%) who met the UCTD criteria had distinct radiological and pathological features of NSIP, whereas the frequency of the patient with IPF/UIP who met the UCTD was unusual (6%).[11] Also, Corte et al. demonstrated that UCTD was present in 31% of NSIP and 13% of IPF.[30] In our study, five of 61 patients (8.2%) diagnosed as IPF at first presentation met the UCTD criteria, suggesting that the UCTD seems to be uncommon manifestation in the patients with IPF. In this study, three out of nine patients (33%) with MPO-ANCA-positive pulmonary fibrosis met the UCTD criteria and two (22%) did the criteria for diagnosis of MPA. MPO-ANCA-positive pulmonary fibrosis may be a phenotype with CTD background among IPF. Our series contained three patients who had prolonged fever and/or myalgia with high MPO-ANCA titers. They did not satisfy the MPA criteria, however the patients were immediately treated with immunosuppressants. This finding suggests that early intervention could prevent the progression of vasculitis.

Homma and colleagues showed that positive MPO-ANCA is an unfavorable prognostic factor in patients with pulmonary fibrosis.[20] In addition, Nozu and colleagues demonstrated that a high titer of ANCA is associated with a poor prognosis as well as the occurrence of MPA.[13] In the present study, 5 of 6 patients with MPO-ANCA titers over 100 EU died, while 2 of 3 patients with titers below 100 EU survived, which agreed with the data by Nozu et al.[13] On the while, the cumulative survival rate of MPO-ANCA-positive cases after diagnosis of IPF was comparable to that of MPO-ANCA-negative IPF patients. However, vasculitis appeared to be well controlled in most cases. Our data indicate that serial measurement of MPO-ANCA and early intervention may improve the outcome of MPO-ANCA positive pulmonary fibrosis.

This study demonstrated occasional MPO-ANCA positive conversion in IPF patients and among those that show conversion, some develop MPA. BAL eosinophilia and low attenuation areas on HRCT scans may predict the positive conversion of MPO-ANCA. Because the sample size of this study is small, we cannot determine the precise incidence of MPO-ANCA positivity in IPF. However, this follow-up survey is of significant value to show that MPO-ANCA conversion can occur in the IPF patients in a certain ratio. In the future, further prospective study should be required.

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Author contribution

Conception and design: M Ando, E Miyazaki, T Ishii, T Ito, S Nureki, T Kumamoto. Analysis and interpretation: M Ando, E Miyazaki, Y Mukai, R Takenaka, T Ueno, T Ito, S Nureki, T Kumamoto. Drafting the manuscript for important intellectual content: M Ando, E Miyazaki, T Kumamoto. M. Ando takes responsibility for the entire manuscript as a whole, from inception to published article.

Conflict of interest

None of authors have any conflicts of interest pertinent to this research to disclose.

References

1. Jennette JC, Wilkman AS, Falk RJ. Anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. *Am J Pathol* 1989;**135**:921—30.
2. van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, et al. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1985;**23**:425—9.
3. Beer DJ. ANCAs aweigh. *Am Rev Respir Dis* 1992;**146**:1128—30.
4. Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med* 1997;**337**:1512—23.
5. Nolle B, Specks U, Ludemann J, et al. Anticytoplasmic autoantibodies: their immunodiagnostic value in Wegener granulomatosis. *Ann Intern Med* 1989;**111**:28—40.
6. Tzelepis GE, Kokosi M, Tzioufas A, et al. Prevalence and outcome of pulmonary fibrosis in microscopic polyangiitis. *Eur Respir J* 2010;**36**:116—21.
7. Gal AA, Velasquez A. Antineutrophil cytoplasmic autoantibody in the absence of Wegener's granulomatosis or microscopic polyangiitis: implications for the surgical pathologist. *Mod Pathol* 2002;**15**:197—204.
8. Gaudin PB, Askin FB, Falk RJ, et al. The pathologic spectrum of pulmonary lesions in patients with anti-neutrophil cytoplasmic autoantibodies specific for anti-proteinase 3 and anti-myeloperoxidase. *Am J Clin Pathol* 1995;**104**:7—16.
9. Nada AK, Torres VE, Ryu JH, et al. Pulmonary fibrosis as an unusual clinical manifestation of a pulmonary-renal vasculitis in elderly patients. *Mayo Clin Proc* 1990;**65**:847—56.
10. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med* 2002;**165**:277—304.
11. Kinder BW, Collard HR, Koth L, et al. Idiopathic nonspecific interstitial pneumonia: lung manifestation of undifferentiated connective tissue disease? *Am J Respir Crit Care Med* 2007; **176**:691—7.
12. Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;**37**:187—92.
13. Nozu T, Kondo M, Suzuki K, et al. A comparison of the clinical features of ANCA-positive and ANCA-negative idiopathic pulmonary fibrosis patients. *Respiration* 2009;**77**:407—15.
14. Becker-Merok A, Nossent JC, Ritland N. Fibrosing alveolitis predating microscopic polyangiitis. *Scand J Rheumatol* 1999; **28**:254—6.
15. Hiromura K, Nojima Y, Kitahara T, et al. Four cases of anti-myeloperoxidase antibody-related rapidly progressive glomerulonephritis during the course of idiopathic pulmonary fibrosis. *Clin Nephrol* 2000;**53**:384—9.
16. Mansi IA, Opran A, Sondhi D, et al. Microscopic polyangiitis presenting as idiopathic pulmonary fibrosis: is anti-neutrophilic cytoplasmic antibody testing indicated? *Am J Med Sci* 2001; **321**:201—2.
17. Eschun GM, Mink SN, Sharma S. Pulmonary interstitial fibrosis as a presenting manifestation in perinuclear antineutrophilic cytoplasmic antibody microscopic polyangiitis. *Chest* 2003; **123**:297—301.
18. Sugiyama Y, Kitamura S. Antineutrophil cytoplasmic antibodies in diffuse panbronchiolitis. *Respiration* 1999;**66**:233—5.
19. Sitara D, Hoffbrand BI. Chronic bronchial suppuration and antineutrophil cytoplasmic antibody (ANCA) positive systemic vasculitis. *Postgrad Med J* 1990;**66**:669—71.
20. Homma S, Matsushita H, Nakata K. Pulmonary fibrosis in myeloperoxidase antineutrophil cytoplasmic antibody-associated vasculitides. *Respirology* 2004;**9**:190—6.
21. Ando Y, Okada F, Matsumoto S, et al. Thoracic manifestation of myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA)-related disease. CT findings in 51 patients. *J Comput Assist Tomogr* 2004;**28**:710—6.
22. Foulon G, Delaval P, Valeyre D, et al. ANCA-associated lung fibrosis: analysis of 17 patients. *Respir Med* 2008;**102**:1392—8.
23. Sessa A, Meroni M, Battini G, et al. Cigarette smoking and pauci-immune extracapillary glomerulonephritis with ANCA-associated idiopathic systemic vasculitis. A retrospective study. *Contrib Nephrol* 2000;**130**:103—8.
24. Churg A, Zay K, Shay S, et al. Acute cigarette smoke-induced connective tissue breakdown requires both neutrophils and macrophage metalloelastase in mice. *Am J Respir Cell Mol Biol* 2002;**27**:368—74.
25. Bodolay E, Csiki Z, Szekanecz Z, et al. Five-year follow-up of 665 Hungarian patients with undifferentiated connective tissue disease (UCTD). *Clin Exp Rheumatol* 2003;**21**: 313—20.
26. Williams HJ, Alarcon GS, Joks R, et al. Early undifferentiated connective tissue disease (CTD). VI. An inception cohort after 10 years: disease remissions and changes in diagnoses in well established and undifferentiated CTD. *J Rheumatol* 1999;**26**: 816—25.
27. Danieli MG, Fraticelli P, Salvi A, et al. Undifferentiated connective tissue disease: natural history and evolution into definite CTD assessed in 84 patients initially diagnosed as early UCTD. *Clin Rheumatol* 1998;**17**:195—201.
28. Mosca M, Tavoni A, Neri R, et al. Undifferentiated connective tissue diseases: the clinical and serological profiles of 91 patients followed for at least 1 year. *Lupus* 1998;**7**:95—100.
29. Clegg DO, Williams HJ, Singer JZ, et al. Early undifferentiated connective tissue disease. II. The frequency of circulating antinuclear antibodies in patients with early rheumatic diseases. *J Rheumatol* 1991;**18**:1340—3.
30. Corte TJ, Copley SJ, Desai SR, et al. Significance of connective tissue disease features in idiopathic interstitial pneumonia. *Eur Respir J* 2012;**39**:661—8.