Significance of antineutrophil cytoplasmic antibody positivity in patients with systemic sclerosis: a single-centre pilot study in Korea

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ABSTRACT

Objective. *We investigated whether antineutrophil cytoplasmic antibody (ANCA) positivity at diagnosis may be associated with the cross-sectional clinical features at diagnosis and predicting all-cause mortality during follow-up in Korean patients with systemic sclerosis (SSc). In addition, we assessed the incidence of SSc and ANCA-associated vasculitis (AAV) overlap syndrome in patients with ANCA positivity.*

Methods. *We retrospectively reviewed the clinical and laboratory features through the medical records of 177 SSc patients who fulfilled the inclusion and exclusion criteria. SSc was classified by the 2013 American College of Rheumatology/European League Against Rheumatism classification criteria. AAV was classified by the 2007 European Medicine Agency algorithms and the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides.*

Results. *The median age was 52 years, and 23 patients were males. The detection rate of ANCA in Korean patients with SSc was 20.3%. Unlike a previous study, ANCA positivity at diagnosis was significantly associated with neither the cross-sectional clinical and laboratory variables at diagnosis nor the rate of all-cause mortality during follow-up in Korean patients with SSc. However, three female patients (8.3%) with ANCA could be classified as having microscopic polyangiitis (MPA) during follow-up.*

Conclusion. *No significant associations of ANCA positivity with the cross-section clinical features or all-cause mortality during follow-up were observed in this study. But, given that 3 of 36 SSc patients with ANCA were classified as having AAV based on the histological confirmation, we suggest that physicians should consider recommending a biopsy when AAV is strongly suspected in SSc patients with ANCA.*

Introduction

Systemic sclerosis (SSc) is a systemic autoimmune disease characterised by vasculopathy and fibrosis of the skin and internal organs. The common systemic complications of SSc include pulmonary arterial hypertension, interstitial lung disease, and gastrointestinal symptoms (1). The pathogenesis of this disease includes development of vascular, immune system, and extracellular membrane abnormalities, which are mediated by genetic and environmental factors. The immune system abnormalities may originate from the dysregulation of T cell subsets, B cells, and innate immunity-related autoreactive cells and cytokines, as well as the alteration of the interferon signature (2). The recent classification criteria for SSc were proposed by the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) in 2013. According to the 2013 ACR/EULAR criteria, a patient is given one point each for the presence of skin thickening, fingertip lesions, abnormal nailfold capillaries, pulmonary arterial hypertension and/or interstitial lung disease, Raynaud's phenomenon, and SSc-related autoantibodies, and a total score ≥9 is sufficient to establish a diagnosis of SSc (3). In particular, the criterion for SSc-related autoantibodies includes three antibodies, namely anti-centromere, anti-topoisomerase I, and anti-ribonucleic acid (RNA) polymerase III antibodies (2, 3). Anti-centromere and anti-topoisomerase I antibodies are found in more than 50% of patients with SSc. Anti-centromere antibodies are associated with limited SSc and pulmonary arterial hyperten-

sion, and anti-topoisomerase I antibodies are associated with diffuse SSc and interstitial lung disease (4). In addition to these autoantibodies, circulating autoantibodies against the muscarinic acetylcholine receptor M3, platelet-derived growth factor, endothelin-1, and angiotensin II have been introduced in patients with SSc and have been reported to trigger receptor activation and accelerate the fibrotic process (5).

A recent study reported the clinical significance of the presence of antineutrophil cytoplasmic antibodies (ANCA) in patients with SSc. ANCA was detected in 8.9% of patients with SSc; of these, three patients were eventually classified as having ANCA-associated vasculitis (AAV). ANCA positivity, particularly proteinase 3 (PR3)-ANCA positivity, was significantly associated with interstitial lung disease and pulmonary embolism. Furthermore, ANCA positivity was found to be an independent predictor of all-cause mortality in patients with SSc during follow-up. Therefore, the authors suggested performing ANCA testing to identify patients with poor prognosis (6). We speculate whether these results could be applied to all patients with SSc or whether these results would vary based on ethnic differences. However, no study has investigated the clinical implications of ANCA positivity in Korean patients with SSc. Hence, in this study, we investigated whether ANCA positivity at diagnosis may be associated with the cross-sectional clinical features at diagnosis and predicting all-cause mortality during follow-up in Korean patients with SSc. In addition, we assessed the incidence of SSc and AAV overlap syndrome in patients with ANCA positivity in order to cope with the poor prognosis of AAV without proper treatment due to a prejudice of SSc diagnosis (7).

Patients and methods

Patients

We obtained a list of 232 patients with SSc using the clinical data repository system of our hospital. They were diagnosed with SSc based on either the 1980 ACR criteria or the 2013 ACR/EULAR criteria (3, 8). The following patients were included: i) patients who were first classified at the Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, Severance Hospital, from June 2004 to November 2020; ii) patients who were reclassified as having SSc according to the 2013 ACR/EULAR classification criteria for SSc (a total score ≥9) (3); iii) patients who had medical records with sufficient details to allow collection of clinical and laboratory data, including autoantibody findings; iv) patients who had results of both ANCA tests, indirect immunofluorescence assay for perinuclear (P)-ANCA and cytoplasmic (C)-ANCA and antigen-specific immunoassays for myeloperoxidase (MPO)-ANCA and PR3-ANCA. Immunoassays were used as the primary screening method for diagnosing ANCA positivity. However, given that the antigen-specific immunoassays have high specificity but low sensitivity due to a narrow detection range, a positive P-ANCA or C-ANCA indirect immunofluorescence assay finding was not ignored in this study (9). The following patients were excluded: i) patients who had serious medical conditions, such as malignancies and infectious diseases, which could affect ANCA positivity; ii) patients who had autoimmune diseases, such as inflammatory bowel disease or primary sclerosing cholangitis, that could result in false ANCA positivity findings (10); iv) patients who were on medications, such as propylthiouracil (PTU), which could cause false ANCA positivity findings (11); v) patients who had not been followed up for more than 3 months after diagnosis.

We retrospectively reviewed the medical records of 232 patients with SSc. Of these patients, 10 did not fulfil the 2013 criteria (3). Of the remaining 222 patients, 32 were excluded due to lack of ANCA test results at diagnosis. Of the 190 patients, four were excluded due to concomitant malignancies at diagnosis. Of the 186 patients, three, two, and two patients were excluded due to Crohn's disease, ulcerative colitis, and primary sclerosing cholangitis at diagnosis, respectively. Of the 179 patients, two patients were excluded as they were on PTU. Finally, 177 patients with SSc were included in the study. The present study was approved by the Institutional Review Board (IRB) of Severance Hospital (Seoul, Korea; IRB No. 4-2021-0221). Given the retrospective design of the study and the use of anonymised patient data, the requirement for written informed consent was waived (Fig. 1).

Clinical and laboratory features

Age and sex were collected as demographic data. Based on the 2013 criteria, SSc-related clinical manifestations were reviewed, and the number of patients presenting with each item was counted. In addition, the total score as per the 2013 criteria was calculated. The anti-RNA polymerase III test is not available at our hospital and is not routinely performed. Therefore, the subitem anti-RNA polymerase III antibody was not included in this study. The results of both ANCA tests (indirect immunofluorescence assay and antigen-specific immunoassay), white blood cell and platelet counts, and levels of haemoglobin, blood urea nitrogen, serum creatinine, aspartate aminotransferase, alanine aminotransferase, total protein, and serum albumin were assessed. In terms of variables during the follow-up, all-cause mortality was defined as a poor outcome, and the number of deceased patients was counted. The follow-up period was defined as the period from the time of diagnosis of SSc to death in deceased patients and from the time of diagnosis of SSc to the last visit in surviving patients. A tissue biopsy was not routinely performed in SSc patients but was performed when AAV is strongly suspected in SSc patients with ANCA.

Interstitial lung disease

Interstitial lung disease was diagnosed by high-resolution computed tomography (HRCT) (12). Interstitial lung disease was also subdivided into two groups, idiopathic pulmonary fibrosis (IPF) and non-IPF. Non-IPF includes other types of interstitial lung disease except for IPF such as non-specific interstitial pneumonia, cryptogenic organising pneumonia, and unclassifiable lung lesions on HRCT (13). On the other hand, the severity of IPF was assessed by forced vital capacity (FVC)

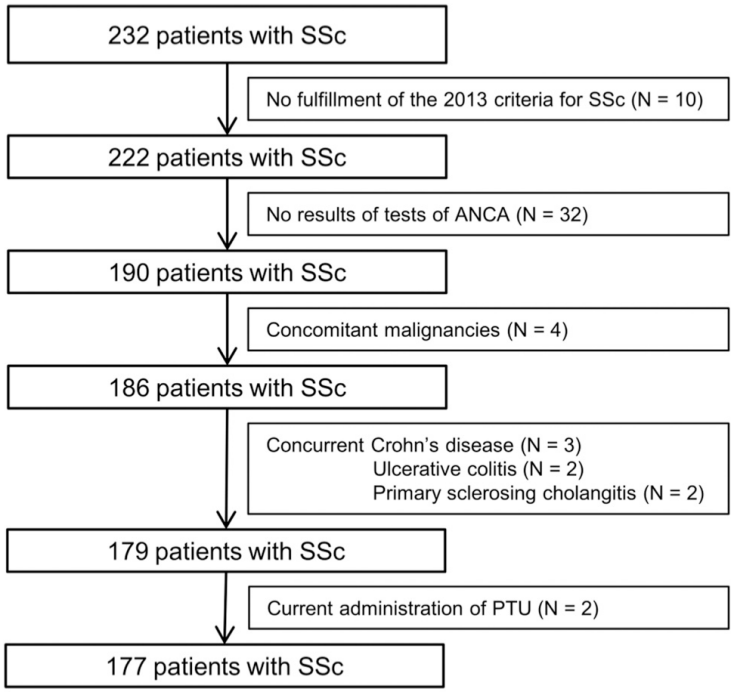

Fig. 1. The algorithm of selecting patients with SSc by the inclusion and exclusion criteria. SSc: systemic sclerosis; ANCA: antineutrophil cytoplasmic antibody; PTU: propylthiouracil.

and diffusing capacity of the lung for carbon monoxide (DLCO) (14).

Classification criteria for AAV

In this study, the algorithms for classification of AAV proposed by the European Medicine Agency in 2007 (the 2007 EMA algorithm) and the revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides (CHCC) established in 2012 (the 2012 definitions) were applied to all SSc patients with ANCA (15, 16).

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, v. 25 (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as medians with interquartile ranges, whereas categorical variables are expressed as numbers (percentages). Significant differences between two categorical variables were analysed using chi-square and Fisher's exact tests. Significant differences between two continuous variables were compared using a Mann-Whitney U-test. Comparison of cumulative survival rates between the two groups was analysed using the Kaplan-Meier method of survival analysis with a log-rank test. The multivariate Cox hazard model using variables with statistical significance in the univariate Cox hazard model was used to obtain the hazard ratios (HRs) during the follow-up duration. *p*-values <0.05 were considered statistically significant.

Results

Characteristics of patients with SSc at diagnosis and during follow-up

In terms of variables at diagnosis, the median age was 52 years, and 23 patients were males. ANCA was detected in 36 patients (20.3%) (MPO-ANCA in 27 patients and PR3-ANCA in 9 patients). The most common SSc-related manifestation was sclerodactyly (59.9%), followed by interstitial lung disease (53.1%), and Raynaud's phenomenon (50.3%). Anti-topoisomerase I and anti-centromere antibodies were detected in 71 (40.1%) and 45 (25.4%) patients, respectively. Nineteen patients (10.7%) died due to various causes during a mean follow-up period of 80.0 months (Table I).

Comparison between SSc patients with and without ANCA

There were no significant differences in the demographic characteristics between the two groups. Among SSc-related manifestations, 69 of 141 (48.9%) patients without ANCA were classified as limited SSc, whereas 23 of 36 patients (63.9%) were diagnosed with limited SSc, however, it did not reach the statistical significance. Patients with SSc exhibited skin thickening and sclerodactyly at similar rates regardless of the presence of ANCA. Unlike the results of a previous study (6), the incidence rate of interstitial lung disease did

Table I. Characteristics of patients with SSc at diagnosis and during follow-up.

Variables	Values
At the time of diagnosis	
Demographic data	
Age (years)	52.0 (19.0)
Male gender (n, (%))	23 (13.0)
SSc-related manifestations	
Skin thickening of the fingers extending MCP joints	65 (36.7)
Sclerodactyly with/without puffy fingers	106 (59.9)
Fingertip lesions	37 (20.9)
Telangiectasia	3 (1.7)
Abnormal nailfold capillaries	26 (14.7)
Pulmonary arterial hypertension	45 (25.4)
Interstitial lung disease	94 (53.1)
Raynaud's phenomenon	89 (50.3)
SSc-related autoantibodies	
Anti-centromere	45 (25.4)
Anti-topoisomerase I	71 (40.1)
Anti-RNA polymerase III	N/A
Total score	11 (3)
ANCA positivity (n, (%))	
ANCA positivity	36 (20.3)
MPO-ANCA (or P-ANCA) positivity	27 (15.3)
PR3-ANCA (or C-ANCA) positivity	9 (5.1)
Laboratory results	
White blood cell count (/mm^3)	6,700.0 (3,700)
Haemoglobin (g/dL)	12.8 (1.8)
Platelet count x (1,000/mm^3)	258.0 (97.5)
Blood urea nitrogen (mg/dL)	12.0 (5.8)
Serum creatinine (mg/dL)	0.7 (0.2)
Aspartate aminotransferase (IU/L)	21.0 (12.0)
Alanine aminotransferase (IU/L)	16.0 (12.0)
Total protein (g/dL)	7.2 (0.9)
Serum albumin (g/dL)	4.2 (0.6)
During follow-up	
Mortality	
All-cause mortality (n, (%))	19 (10.7)
Follow-up duration based on mortality (months)	80.0 (96.0)

Values are expressed as a median (interquartile range, IQR) or n (%).
SSc: systemic sclerosis; ESRD: end-stage renal disease; ANCA: antineutrophil cytoplasmic antibody; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic.

not differ between the two groups. SSc patients without ANCA tended to exhibit abnormal nailfold capillaries and Raynaud's phenomenon more frequently than those with ANCA did (17.0% *vs*. 5.6% and 53.9% *vs*. 36.1%, respectively); however, the difference was not statistically significant. There were no significant differences in the detection rates of anti-centromere and anti-topoisomerase I antibodies between the two groups.

As for the mortality rate, the number of deceased patients was similar regardless of the presence of ANCA (Table II).

Table II. Comparison of the characteristics of patients with SSc based on ANCA positivity.

Variables	SSc patients Without ANCA (n=141)	SSc patients With ANCA (n=36)	*p*-value
At the time of diagnosis			
Demographic data			
Age (years)	52.0 (18.0)	52.5 (22.0)	0.999
Male gender (n, (%))	20 (14.2%)	3 (8.3%)	0.421
SSc-related manifestations			
Diffuse SSc / Limited SSc	72 (51.1) / 69 (48.9)	13 (36.1) / 23 (63.9)	0.109
Skin thickening of the fingers extending MCP joints	50 (35.5%)	15 (41.7%)	0.491
Sclerodactyly with/without puffy fingers	86 (61.0%)	20 (55.6%)	0.552
Fingertip lesions	31 (22.0%)	6 (16.7%)	0.484
Telangiectasia	2 (1.4%)	1 (2.8%)	0.497
Abnormal nailfold capillaries	24 (17.0%)	2 (5.6%)	0.113
Pulmonary arterial hypertension	36 (25.5%)	9 (25%)	0.948
Interstitial lung disease	74 (52.5%)	20 (55.6%)	0.742
Raynaud's phenomenon	76 (53.9%)	13 (36.1%)	0.057
SSc-related autoantibodies			
Anti-centromere	37 (26.2%)	8 (22.2%)	0.621
Anti-topoisomerase I	54 (38.3%)	17 (47.2%)	0.330
Total score	11 (3.0)	11 (3.0)	0.866
Laboratory results			
White blood cell count (/mm^3)	6.85 (4.0)	6.85 (4.0)	0.834
Haemoglobin (g/dL)	12.6 (1.5)	12.6 (1.5)	0.207
Platelet count (x1,000/mm^3)	257 (126.3)	257 (126.3)	0.331
Blood urea nitrogen (mg/dL)	11.2 (6.2)	11.2 (6.2)	0.259
Serum creatinine (mg/dL)	0.7 (0.2)	0.7 (0.2)	0.879
Aspartate aminotransferase (IU/L)	20.5 (13.5)	20.5 (13.5)	0.686
Alanine aminotransferase (IU/L)	16.5 (13.5)	16.5 (13.5)	0.456
Total protein (g/dL)	7.3 (0.7)	7.3 (0.7)	0.476
Serum creatinine (g/dL)	4.1 (0.5)	4.1 (0.5)	0.481
During follow-up			
Mortality			
All-cause mortality (n, (%))	14 (9.9%)	5 (13.9%)	0.547

Values are expressed as a median (interquartile range, IQR) or n (%).
SSc: systemic sclerosis; ANCA: antineutrophil cytoplasmic antibody; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic.

Comparison between 94 SSc patients with interstitial lung disease based on ANCA positivity

We selected 94 SSc patients with interstitial lung disease and compared the chest CT findings and pulmonary function test results at diagnosis and all-cause mortality during follow-up. Of 20 patients with SSc-ILD with ANCA, 12 patients (60%) exhibited a pattern of IPF. However, no significant differences in the proportion of IPF, FVC, and DLCO between the two groups were observed. In addition, all-cause mortality did not differ in SSc patients with interstitial lung disease according to the presence of ANCA (Supplementary Table S1).

Comparison among SSc patients with MPO-ANCA, those with PR3-ANCA, and those without ANCA

In the first comparison between 141 SSc patients without ANCA and 27 SSc patients with MPO-ANCA, abnormal nailfold capillaries (17.0% *vs*. 3.7%, p=0.083) and Raynaud's phenomenon (53.9% *vs*. 33.3%, p=0.050)were observed in SSc patients without ANCA more often than that in patients with MPO-ANCA; however, these differences were not statistically significant. Conversely, anti-topoisomerase I antibody was detected less often in SSc patients without ANCA than in those with MPO-ANCA (38.3% *vs*. 55.6%, p=0.095) but without significance. There were no differences in the laboratory results and mortality rates between the two groups. In the second comparison between 141 SSc patients without ANCA and nine SSc patients with PR3-ANCA, no significant differences were found in the demographic and SSc-related characteristics between the two groups. Meanwhile, SSc patients without ANCA exhibited significantly elevated white blood cell and platelet counts than those with PR3-ANCA did (6,700.0/mm^3 *vs*. 4,700.0/mm^3, p=0.009 and 250,0000/mm^3 *vs*. 227,000.0/mm^3, p=0.019, respectively). There were no differences in the mortality rates between the two groups. In the last comparison between 27 SSc patients with MPO-ANCA and nine SSc patients with PR3-ANCA, the demographic and SSc-related characteristics between the two groups did not differ. Among the laboratory results, only the white blood cell count was significantly different between those with MPO-ANCA and those with PR3-ANCA (7,200.0/mm^3 *vs*. 4,700.00/mm^3, p=0.007). There were no differences in the mortality rates between the two groups (Table III).

Comparison of the cumulative patient survival rates

On comparing the cumulative patient survival rates between SSc patients without ANCA and those with ANCA, no significant difference was found (p=0.692). Furthermore, on comparing the cumulative survival rates between SSc patients without ANCA, those with MPO-ANCA, and those with PR3-ANCA, no significant difference was found (p=0.254) (Fig. 2).

Classification of AAV

Table III. Comparison of the characteristics of patients with SSc among MPO-ANCA positivity, PR3-ANCA negativity and ANCA negativity.

Variables	SSc patients without ANCA (n=141)	SSc patients with MPO-ANCA (n=27)	SSc patients with PR3-ANCA (n=9)	*p*-value 1	*p*-value 2	*p*-value 3
At the time of diagnosis						
Demographic data						
Age (years)	52.0 (18)	53.0 (23)	51.0 (18)	0.993	0.991	0.985
Male gender (n, (%))	20 (14.2%)	2 (7.4%)	1 (11.1%)	0.534	1.000	1.000
SSc-related manifestations						
Skin thickening of the fingers extending MCP joints	50 (35.5%)	12 (44.4%)	3 (33.3%)	0.376	1.000	0.705
Sclerodactyly with/without puffy fingers	86 (61.0%)	14 (51.9%)	6 (66.7%)	0.375	0.735	0.439
Fingertip lesions	31 (22.0%)	5 (18.5%)	1 (11.1%)	0.802	0.685	1.000
Telangiectasia	2 (1.4%)	0 (0.0%)	1 (11.1%)	1.000	0.170	0.250
Abnormal nailfold capillaries	24 (17.0%)	1 (3.7%)	1 (11.1%)	0.083	1.000	0.443
Pulmonary arterial hypertension	36 (25.5%)	8 (29.6%)	1 (11.1%)	0.657	0.453	0.396
Interstitial lung disease	74 (52.5%)	15 (55.6%)	5 (55.6%)	0.769	1.000	1.000
Raynaud's phenomenon	76 (53.9%)	9 (33.3%)	4 (44.4%)	0.050	0.734	0.693
SSc-related autoantibodies						
Anti-centromere	37 (26.2%)	5 (18.5%)	3 (33.3%)	0.474	0.701	0.384
Anti-topoisomerase I	54 (38.3%)	15 (55.6%)	2 (22.2%)	0.095	0.485	0.128
Total score	11 (3.0)	12 (3.0)	10 (3)	0.821	0.436	0.398
Laboratory results						
White blood cell count (/mm^3)	6,700.0 (3,800.0)	7,200.0 (3,800.0)	4,700.0 (2,600.0)	0.240	0.009	0.007
Haemoglobin (g/dL)	12.8 (1.7)	12.6 (1.5)	12.6 (1.8)	0.337	0.326	0.661
Platelet count (x1,000/mm^3)	258.0 (96.0)	269.0 (114.0)	227.0 (122.0)	0.900	0.019	0.051
Blood urea nitrogen (mg/dL)	12.4 (5.6)	11.2 (6.7)	10.8 (4.9)	0.445	0.291	0.596
Serum creatinine (mg/dL)	0.7 (0.2)	0.7 (0.3)	0.7 (0.2)	0.783	0.861	0.765
Aspartate aminotransferase (IU/L)	21.5 (11.0)	22.0 (14.0)	17.0 (5.5)	0.620	0.074	0.111
Alanine aminotransferase (IU/L)	16.0 (12.0)	17.0 (13.0)	14.0 (13.0)	0.368	0.975	0.558
Total protein (g/dL)	7.2 (0.9)	7.3 (1.0)	7.3 (0.5)	0.479	0.802	0.687
Serum albumin (g/dL)	4.2 (0.6)	4.1 (0.8)	4.3 (0.5)	0.291	0.686	0.429
During follow-up						
Mortality						
All-cause mortality (n, (%))	14 (9.9%)	5 (18.5%)	0 (0%)	0.196	1.000	0.302

Values are expressed as a median (interquartile range, IQR) or n (%).
SSc: systemic sclerosis; ANCA: antineutrophil cytoplasmic antibody; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic.
p-value 1: *p*-value between patients without ANCA and those with MPO-ANCA; *p*-value 2: *p*-value between patients without ANCA and those with PR3-ANCA; *p*-value 3: *p*-value between patients with MPO-ANCA and those with PR3-ANCA.

We applied the 2007 EMA algorithm and the 2012 CHCC definitions to 36 SSc patients with ANCA and found that three female patients (8.3%) could be classified as having microscopic polyangiitis (MPA) as well. Patient 1 was classified as having MPA based on a histological examination of the lungs conducted 7 years after the diagnosis of SSc. The histological findings were necrotising vasculitis in small vessels without evidence of granuloma, eosinophilic infiltrates, or haemosiderin-laden macrophages. Patient 2 was classified as having MPA based on a histological examination of the kidneys along with otorhinolaryngologic and pulmonary manifestations, a year after the diagnosis of SSc. The histological finding was pauci-immune crescentic glomerulonephritis, which was corresponding with AAV. Patient 3 was classified as having MPA based on a histological examination of the nerves in the lower extremity, in addition to otorhinolaryngologic and pulmonary manifestations. The histological findings were lymphocytic infiltration in the epineural vessels with fibrinoid necrosis (Table IV).

Discussion

In this study, we investigated the clinical implications of the presence of ANCA in Korean patients with SSc. We compared the results with those of a previous study and observed five major findings (6). First, the detection rate of ANCA in Korean patients with SSc was 20.3%, which was higher than that reported in the previous study (8.9%). Second, ANCA positivity at diagnosis was not associated with the cross-sectional presence of interstitial lung disease in Korean patients with SSc, unlike in the previous study. Third, ANCA positivity at diagnosis was not a valuable predictor of all-cause mortality during follow-up in Korean patients with SSc, unlike in the previous study. Fourth, in addition to ANCA positivity, PR3-ANCA (or C-ANCA) was not significantly associated with cross-sectional interstitial lung disease or all-cause mortality during follow-up in Korean patients with SSc, unlike in the previous study. Last, three female patients (8.3%) with ANCA could be classified as having microscopic polyangiitis (MPA). Therefore, we concluded that

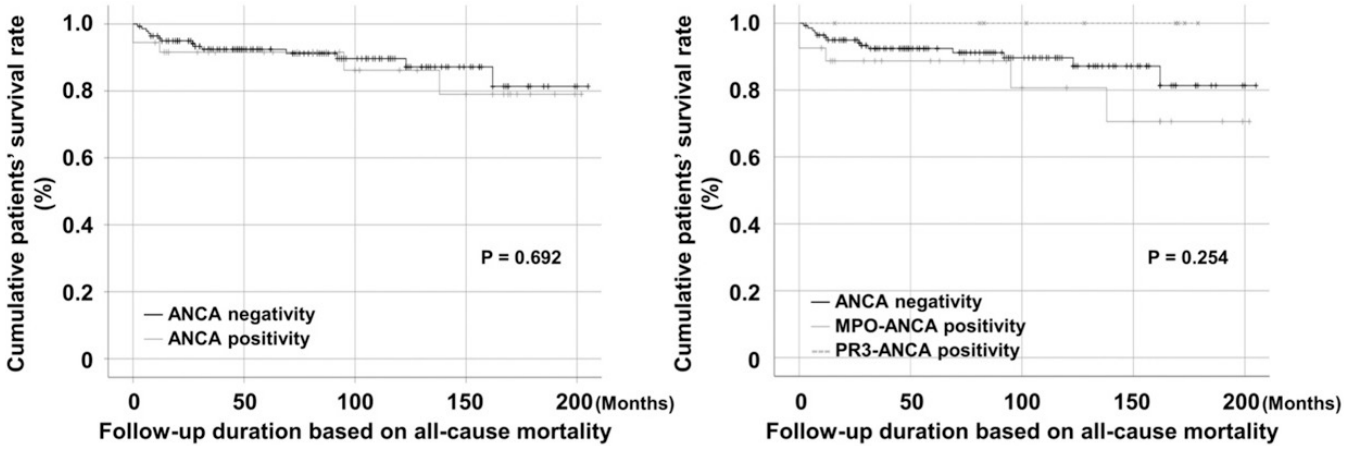

Fig. 2. Comparison of the cumulative patient survival rates.
There were no significant differences in the cumulative patients' survival rates between SSc patients with ANCA and those without ANCA or among SSc patients without ANCA, those with MPO-ANCA and those with PR3-ANCA.
SSc: systemic sclerosis; ANCA: antineutrophil cytoplasmic antibody; MPO: myeloperoxidase; PR3: proteinase 3.

Table IV. Characteristics of patients with SSc and AAV (overlap syndrome).

		At the time of diagnosis of SSc			At the time of diagnosis of AAV					
Patient	Age	Date	Gender	SSc-related manifestations	Age	Date	AAV subtype	ANCA	AAV-related manifestations	BVAS/FFS
1	52	Feb 2011	Female	Sclerodactyly ILD Anti-topoisomerase I	59	March 2018	MPA	MPO-ANCA	General Lung (Bx)	6/1
2	36	May 2014	Female	Sclerodactyly ILD RP	37	May 2015	MPA	MPO-ANCA	General ENT Lung Kidney (Bx)	16/1
3	63	June 2010	Female	Sclerodactyly Fingertip lesions ILD RP	66	May 2013	MPA	PR3-ANCA	General ENT Lung PNS (Bx)	6/2

SSc: systemic sclerosis; AAV: ANCA-associated vasculitis; ANCA: antineutrophil cytoplasmic antibody; BVAS: Birmingham vasculitis activity score; FFS: five factor score; ILD: interstitial lung disease; MPA: microscopic polyangiitis; MPO: myeloperoxidase; Bx: biopsy; RP: Raynaud's phenomenon; ENT: ear nose throat; PR3: proteinase 3; PNS: peripheral nervous system.

ANCA positivity did not contribute to the cross-sectional clinical manifestations at diagnosis and all-cause mortality during follow-up in Korean patients with SSc. However, ANCA positivity seemed to be associated with AAV development in patients with SSc.

A previous study reported that 8.9% of patients with SSc had ANCA (3), whereas in our study 20.3% of patients with SSc had ANCA. Moreover, in our study, the proportion of patients with SSc and AAV overlap syndrome was significantly higher than that in previous studies (2.6% *vs*. 0.23-1.6%) (6, 17). The same SSc and AAV classification criteria were used in the previous and current studies. We hypothesise that the differences in results could be attributed to ethnic differences and the different patterns of autoreactive B cell functions. In terms of ethnicity and geography, the ethnicity of the cohorts was quite different. In the previous study, most patients were Caucasian (90.4%) and only 4.5% of patients were Asian (6); whereas, in our study, all patients were Korean. Although the number of studies is small, ethnic differences cannot be ignored. In terms of autoantibodies related to SSc, patients in the previous study exhibited a higher detection rate of anti-centromere antibodies (47.0% *vs*. 25.4%) and a lower detection rate of anti-topoisomerase I antibodies (13.9% *vs*. 40.1%) compared with those of patients in the present study. Although a direct comparative analysis of two studies is impossible, these results imply that the pattern of dysregulation in autoreactive B cell functions might differ according to ethnicities, which could explain the differences in the detection rate of ANCA and the proportion of patients classified as having AAV between the two studies. On the other hand, the clinical features and disease course are known to be affected by sex and ethnic differences (18, 19). However, between the previous study and our study, there was no difference among females (86.3% *vs*. 87.0%). Therefore, female sex did not seem to be an important player.

What is the reason the majority of SSc patients with ANCA did not exhibit the clinical features of AAV? We have two hypotheses. One is that since clinical manifestations of AAV might not be as distinct as fibrosis-related symptoms in major organs in SSc patients such as interstitial lung disease and decreased peristalsis in the gastrointestinal tract, AAV could be masked by SSc. Three SSc patients who were diagnosed with overlap syndrome clearly exhibited AAV-related clinical manifestations, leading to AAV classification (Table IV). The other is that ANCA positivity might be unclassifiable ANCAs (or ANCA false positive) as was previously reported in various rheumatic diseases, especially in autoimmune connective tissue diseases (20). But these hypotheses could not be proven due to a limitation of a retrospective design of this study.

Unlike the previous study (6), in this study, the mortality rate did not differ between patients with ANCA and those without ANCA in the cross-sectional comparison. In addition, neither ANCA positivity nor MPO-ANCA positivity was significantly associated with all-cause mortality during follow-up as per the Kaplan-Meier survival analysis. To identify all-cause mortality predictors other than ANCA positivity in patients with SSc, we conducted univariate and multivariate Cox hazards analyses as described in Supplementary Table S2. In the univariate analysis using clinical variables at diagnosis, age, telangiectasia, and pulmonary arterial hypertension were significantly associated with all-cause mortality during follow-up. Among the laboratory results at diagnosis, white blood cell count and levels of haemoglobin, blood urea nitrogen, serum creatinine, and serum albumin were significantly associated with all-cause mortality during follow-up. In the multivariate analysis, which did not include laboratory variables with statistical significance in the univariate analysis, age (HR 1.092, 95% confidence interval (CI) 1.047, 1.139) and pulmonary arterial hypertension (HR 5.104, 95% CI 2.000, 13.022) were independent predictors of all-cause mortality during follow-up in patients with SSc. In the multivariate analysis including laboratory variables with statistical significance in the univariate analysis, the independent predictors of all-cause mortality during follow-up in patients with SSc were age (HR 1.081, 95% CI 1.032, 1.132), pulmonary arterial hypertension (HR 5.393, 95% CI 1.820, 15.981), and white blood cell count (HR 1.188, 95% CI 1.013, 1.394). However, interstitial lung disease was not an independent predictor of all-cause mortality in the present study.

We first investigated the clinical significance of ANCA positivity in Korean patients with SSc. Given the effect of ethnic differences on study findings, this study including only Korean patients with SSc has the advantage that it could provide information on the clinical implications of ANCA in Asian patients with SSc. These findings can be used generalised to patients of Asian descent in different countries. However, this study has several limitations. First, as it was a single-centre study, the number of patients was not large enough to be representative of all Korean patients with SSc. Second, owing to the retrospective nature of this research, we could not rule out the possibility of other accompanying autoimmune diseases or chronic diseases affecting both clinical manifestations and all-cause mortality. In addition, due to a limitation of a retrospective design, we could neither clarify the association of MPO-ANCA and interstitial lung disease in 14 patients who had both MPO-ANCA and interstitial lung disease but were not diagnosed with AAV nor describe the risk factors of interstitial lung disease and full parameters of pulmonary function test in SSc patients with MPO-ANCA (21).

Third, most drugs administered in other medical institutions could be viewed through the Korean Drug Utilisation Review system but could not be completely accounted for. Fourth, pulmonary thromboembolism was not documented in the medical records of all patients; therefore, its incidence or influence on all-cause mortality was not assessed. Lastly, it was impossible to apply the 2007 EMA algorithm and the 2012 CHCC definitions for classification of AAV to all SSc patients without ANCA owing to insufficient information in the medical records. For this reason, patients with ANCA-negative AAV may have been missed. A nationwide study with a larger number of patients with SSc will provide more reliable information on the clinical implications of ANCA positivity in patients with SSc.

In conclusion, ANCA positivity at diagnosis was significantly associated with neither the cross-sectional clinical and laboratory variables at diagnosis nor the rate of all-cause mortality in Korean patients with SSc. In contrast, 3 of 36 SSc patients with ANCA were classified as having AAV based on histological confirmation. Therefore, we suggest that physicians should consider recommending a biopsy when alveolar haemorrhage, glomerulonephritis, and peripheral neuropathy are strongly suspected in SSc patients with ANCA.

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