The Elevation of IgG Levels Is a Serological Indicator for Pulmonary Fibrosis in Systemic Sclerosis with Anti-topoisomerase I Antibodies and Those with Anticentromere Antibody

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The protocol was approved by the Kanazawa University School of Medicine, Kanazawa University Hospital, and Nagasaki University Hospital, and all study subjects provided informed consent according to the Declaration of Helsinki.

Summary

It is unclear that any clinical and laboratory features associate with pulmonary fibrosis (PF) in systemic sclerosis (SSc). The clinical and laboratory features associated with PF were assessed using the database of 29 SSc patients with anti-topo I antibodies and 68 SSc patients with anticentromere antibody (ACA). Clinical features were not associated with the incidence of PF in SSc patients with anti-topo I antibodies, although severe skin sclerosis was correlated with the presence of PF in patients with ACA. Serum IgG levels were frequently elevated in SSc patients with PF. Furthermore, serum IgG levels in patients with PF were significantly higher than those in patients without PF. Furthermore, serum IgG levels were negatively correlated with %VC and %DLco. In addition, serum IgG levels were correlated with serum IL-6. Thus, Serum IgG levels associated with PF in SSc patients with anti-topo I antibodies and those with ACA.

Systemic sclerosis (SSc) is a connective tissue disorder characterized by sclerotic changes in skin and internal organs. The severity and prognosis of SSc are highly variable. Mortality rate depends on the involvement of skin, kidney, heart, or lung. Pulmonary fibrosis (PF) occurs in more than 50% of SSc patients and 80% of those with anti-topoisomerase I (anti-topo I) antibodies (Abs) [1]. Since PF is one of the major course of death in SSc patients, the evaluation and prediction of PF is very important [2]. Many patients with SSc were initially referred to dermatologists, who are required to make the initial diagnosis and to predict the involvement of internal organs, since Raynaud's phenomenon and skin sclerosis are the earliest involvements in most SSc patients. The objectives in this study were to estimate the incidence of PF in Japanese SSc patients and quantify the influence of the clinical or laboratory features.

Report

Twenty-nine SSc patients with anti-topo I Abs and sixty-eight those with anticentromere antibody (ACA), who were referred to Dermatology, Kanazawa University since 1,999 to 2,005 were examined. All patients fulfilled the criteria for SSc [3]. These patients were between 2-73 years old (mean age 47). The patients with Sjögren syndrome were excluded. The disease duration was 8.2 ± 11.1 years. None had received immunosuppressive therapy, and had a recent history of infection or other inflammatory diseases. Healthy control sera were obtained from 30 age and sex matched volunteers. Organ system involvement was defined as described previously [4]. The protocol was approved by the Kanazawa University School of Medicine, Kanazawa University Hospital, and Nagasaki University Hospital, and all study subjects provided informed consent according to the Declaration of Helsinki. Serum IgG levels were quantified by standard immunoturbidimetry. Normal range was 800-1774 mg/dl. Mann-Whitney U test for comparison of IgG levels, Fisher's exact probability test for comparison of frequencies, Bonferroni's test for multiple comparisons, and Spearman's rank correlation coefficient for examining the relationship between two continuous variables were used. A p value less than 0.05 was considered statistically significant.

Patients with anti-topo I Abs were divided according to the presence of PF (Table 1). Severe skin involvement, including high modified Rodnan total skin thickness score (TSS), did not correlate with PF. The presence of PF was not associated with the organ involvement. Regarding serological abnormalities, serum IgG levels were more frequently elevated in sera from SSc patients with PF than those from patients without PF (70% vs 0%, $p<0.05$, Table 1). Furthermore, serum IgG levels in patients with PF (2070 ±

511 mg/dl) were significantly higher than those in patients without PF and controls (1504 ± 147 mg/dl, $p<0.05$ and 1530 ± 132 mg/dl, $p<0.03$, respectively; Figure 1). Serum IgG levels were negatively correlated with %VC and %DLco ($r=-0.47$, $p<0.03$ and $r=-0.59$, $p<0.003$, respectively; Figure 2). All patients without PF showed normal IgG levels and showed no complication of PF during follow-up (5.8 ± 2.2 years). Thus, elevated serum IgG levels were associated with PF in patients with anti-topo I Abs.

We also examined patients with ACA. TSS correlated with the presence of PF (Table 1). Serum IgG levels were more frequently elevated in sera from SSc patients with PF than those in sera from patients without PF (75% vs 0%, $p<0.05$, Table 1). Furthermore, serum IgG levels in patients with PF (2032 ± 201 mg/dl) were significantly higher than those in patients without PF and controls (1403 ± 34 mg/dl, $p<0.05$ and 1530 ± 132 mg/dl, $p<0.05$, respectively; Figure 1).

IL-6 promotes the maturation of B lymphocytes and the production of immunoglobulins. Therefore, the relationship between serum IgG levels and IL-6 levels was analyzed in SSc patients positive for anti-topo I Abs, who were followed more than three years. They included 10 out of 29 patients and between 2-72 years old (mean age 47). Specific ELISA kits (R&D Systems, Minneapolis, MN, USA) were used for measuring serum IL-6 levels (detection limit: 0.7 pg/ml). Cut-off value was 7.91 pg/dl [5]. Serum IgG levels were correlated positively with IL-6 levels ($p<0.03$, $r=0.69$, Figure 3).

These results suggest that serum IgG levels can be an indicator for PF. In an anti-topo I positive group and ACA positive group, serum IgG levels in patients with PF were significantly higher than those without PF. However, there was no difference in patients with anti-U1RNP antibodies or those with other SSc-associated antibodies. Since

the current study does not include sufficient number of patients, it should be addressed in the future with a large number of SSc whether relationship between IgG elevation.

This is the first report that suggests the clinical significance of serum IgG levels in SSc patients with anti-topo I Abs. However, there are limitations in this study: relatively small number of SSc patients with anti-topo I Abs were included; and serial studies are not included. Nonetheless, several observations back up the results. First, the change of serum IgG levels associated with the change of pulmonary function tests in patients with SSc [6]. Secondly, the secretion of IL-6, which can induce B cell activation, from peripheral blood monocytes of SSc patients with PF is higher than that of SSc patients without PF [7]. Third, serum IgG levels were correlated with serum IL-6 levels in SSc patients.[5]. Fourth observation is that IgG levels in BAL fluid were elevated in SSc patients with active PF [8]. In addition, plasma cell infiltration into lung is the earliest abnormalities in PF associated with SSc [9]. Since IL-6 associates with B cell activation, the results of this study suggest that B cell activation might be involved in PF in SSc patients. Since the extent of skin sclerosis and involvement of other organs are not always correlated with PF, it seems to be reasonable to evaluate PF in SSc patients by integrating various clinical and laboratory variables. Several serological markers were recently reported to be useful for monitering PF in SSc patients [10]. In addition to these markers, IgG levels may be one of useful tools for evaluation of PF associated with SSc.

REFERENCES

1. Silver RM: Clinical problems: the lungs. Rheum. Dis. Clin. Nor. Am. 1996;22:825-840.
2. Morgan C, Knight C, Lunt M, Black CM, Silman AJ: Predictors of end stage lung disease in a cohort of patients with scleroderma. Ann Rheum Dis 2003;62:146-150.
3. Committee SfSCotARADaTC: Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-590.
4. Sato S, Hamaguchi Y, Hasegawa M, Takehara K: Clinical significance of anti-topoisomerase I antibody levels determined by ELISA in systemic sclerosis. Rheumatology (Oxford) 2001;40:1135-1140.
5. Hasegawa M, Sato S, Fujimoto M, Ihn H, Kikuchi K, Takehara K: Serum levels of interleukin 6 (IL-6), oncostatin M, soluble IL-6 receptor, and soluble gp130 in patients with systemic sclerosis. J. Rheumatol. 1998;25:308-313.
6. Yuhara T, Takemura H, Akama T, Yamane K, Sumida T: The relationship between serum immunoglobulin levels and pulmonary involvement in systemic sclerosis. J Rheumatol 2000;27:1207-1214.
7. Crestani B, Seta N, De Bandt M, Soler P, Rolland C, Dehoux M, Boutten A, Dombret MC, Palazzo E, Kahn MF, et al.: Interleukin 6 secretion by monocytes and alveolar macrophages in systemic sclerosis with lung involvement. Am J Respir Crit Care Med 1994;149:1260-1265.
8. Silver RM, Metcalf JF, Stanley JH, LeRoy EC: Interstitial lung disease in scleroderma. Analysis by bronchoalveolar lavage. Arthritis Rheum 1984;27:1254-1262.

9. Harrison NK, Myers AR, Corrin B, Soosay G, Dewar A, Black CM, Du Bois RM, Turner-Warwick M: Structural features of interstitial lung disease in systemic sclerosis. Am Rev Respir Dis 1991;144:706-713.

10. Yanaba K, Hasegawa M, Hamaguchi Y, Fujimoto M, Takehara K, Sato S: Longitudinal analysis of serum KL-6 levels in patients with systemic sclerosis: association with the activity of pulmonary fibrosis. Clin Exp Rheumatol 2003;21:429-436.

FIGURE LEGENDS

Figure 1.

Serum levels of IgG in patients with SSc and healthy controls. Horizontal bars represent mean levels. A broken line indicates the cut-off value (1774 mg/dl).

Figure 2.

The correlation of serum IgG levels with %VC or %DLco in SSc patients with anti-topo I Abs.

Figure 3.

The correlation of serum IgG levels with serum IL-6 levels in SSc patients with anti-topo I Abs. Serum IL-6 levels were determined by ELISA. The broken lines indicate the cut-off values of serum IgG levels (1774 mg/dl) and serum IL-6 levels (7.9 pg/dl), respectively. Closed circles and open circles indicate SSc patients with PF and those without PF, respectively.

Table 1. Clinical and laboratory data of SSc patients with pulmonary fibrosis

Autoantibody profile	Anti-topoisomerase antibody		Anticentromere antibody	
	SSc patients with PF* (n = 21)	SSc patients without PF (n = 8)	SSc patients with PF* (n = 4)	SSc patients without PF (n = 64)
Sex (male:female)	5:16	1:7	1:3	3:61
Duration (years, mean ± S.D.)	5.7 ± 7.6	4.1 ± 5.6	14.8 ± 15.3	9.7 ± 10.0
Clinical features				
dSSc: lSSc	19: 2	7: 1	4: 0	3: 61
TSS (points, mean ± S.D.)	18.7 ± 10.2	12.5 ± 9.0	12.2 ± 8.3**	5.2 ± 4.6
Pitting scars	14 (67%)	6 (75%)	1 (25%)	12 (19%)
Contracture of phalanges	17 (81%)	3 (38%)	3 (75%)	11 (17%)
Diffuse pigmentation	14 (67%)	3 (38%)	3 (75%)	12 (19%)
Organ involvement				
Decreased %VC	14 (67%)**	1 (13%)	1 (25%)**	1 (2%)
Decreased %DLco	20 (95%)	8 (100%)	2 (50%)	29 (45%)
Esophagus	16 (76%)	7 (88%)	3 (75%)	37 (58%)
Heart	9 (43%)	1 (13%)	0 (0%)	8 (13%)
Kidney	6 (29%)	2 (25%)	0 (0%)	0 (0%)
Joint	8 (38%)	1 (13%)	1 (25%)	14 (22%)
Muscle	3 (14%)	3 (38%)	0 (0%)	5 (8%)
Laboratory findings				
Increased IgG	15 (71%)**	0 (0%)	3 (75%)**	0 (0%)
Elevated ESR	7 (33%)	1 (13%)	2 (50%)	15 (23%)
Elevated CRP	8 (38%)	0 (0%)	2 (50%)	4 (6%)
Anti-topo I Ab (mean ± S.D.)	105 ± 58	111 ± 59		

*PF = pulmonary fibrosis, VC = vital capacity, DLco = diffusion capacity for carbon monoxide, TSS = modified Rodnan total skin thickness score, ESR = erythrocyte sedimentation rates, and CRP = C-reactive protein. Values of clinical features, organ involvement, and laboratory findings represent the number of cases.

**p<0.05 vs. SSc patients without PF

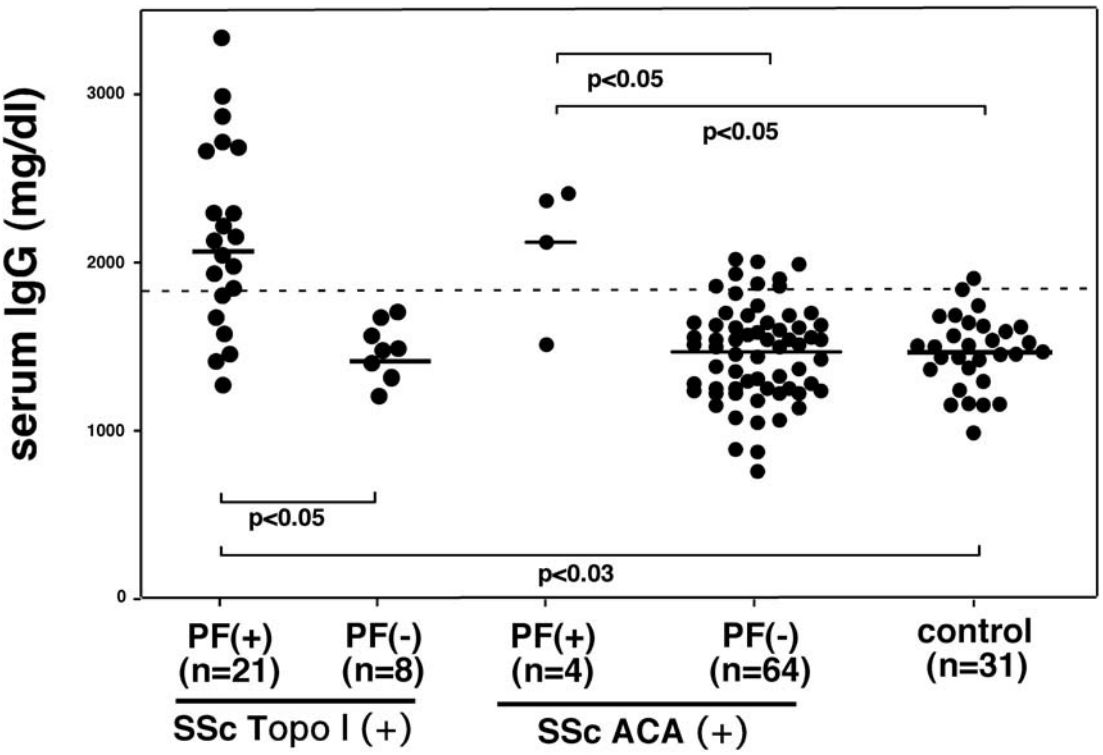

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Figure 1

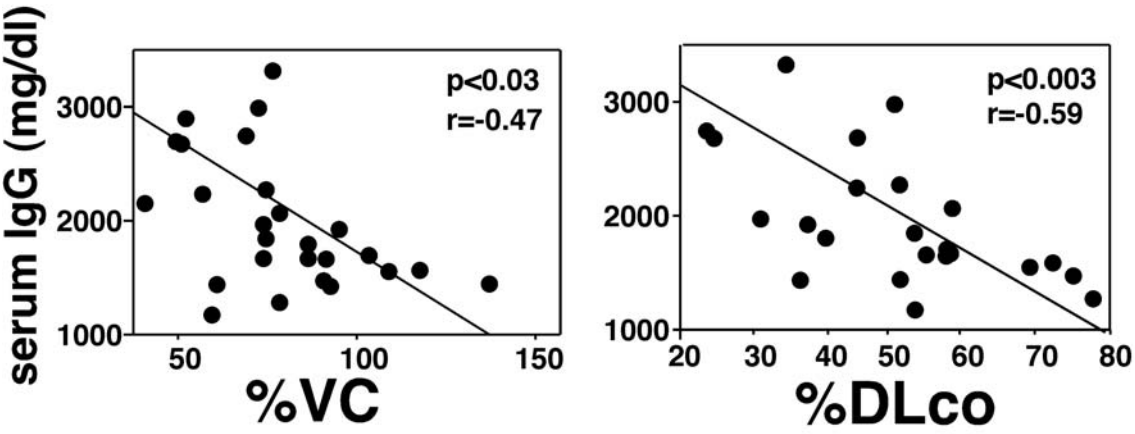

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Figure 2

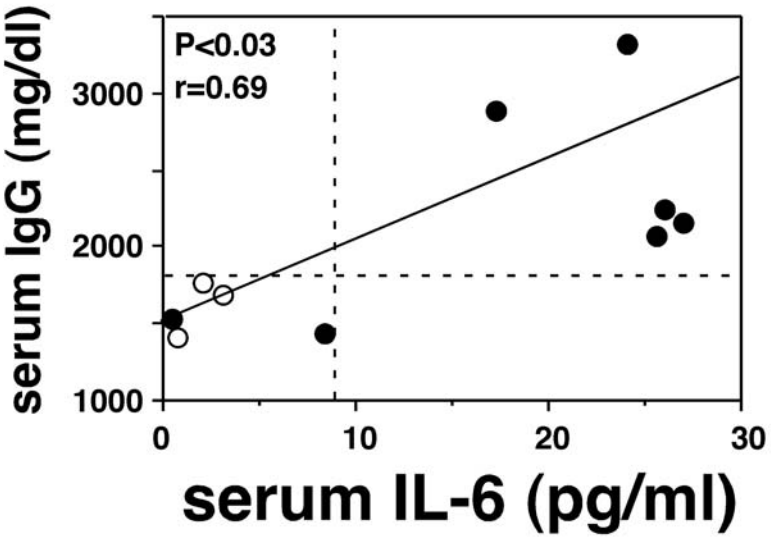

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Figure 3