Anti-Agalactosyl IgG Antibodies in Sera from Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** To determine the prevalence and clinical correlations of anti-agalactosyl IgG antibodies (anti-AG IgG) in patients with systemic sclerosis (SSc).

Methods. Serum samples from patients with limited cutaneous SSc (lSSc; n = 49), diffuse cutaneous SSc (dSSc; n = 21), rheumatoid arthritis (RA; n = 10), systemic lupus erythematosus (SLE; n = 20), and healthy individuals (n = 20) were examined by lectin-enzyme immunoassay using human agalactosyl IgG as antigen.

Results. Anti-AG IgG were detected in 52 (74%) of 70 patients with SSc, which was much higher than the frequency of rheumatoid factor positivity in SSc (16%). Levels of anti-agalactosyl IgG antibodies were significantly higher than in healthy controls or patients with SLE, but lower than patients with RA. Levels of anti-AG IgG in patients with dSSc were significantly higher than in lSSc. SSc patients with anti-topoisomerase I antibodies had significantly higher levels of anti-AG IgG than SSc patients with anticentromere antibodies. Concerning clinical correlation, patients with pulmonary fibrosis showed elevated levels of anti-AG IgG compared to those without pulmonary fibrosis. Patients with decreased %VC or %DLCO showed increased levels of anti-AG IgG. Elevated levels of anti-AG IgG were associated with the presence of contracture of phalanges or cutaneous calcinosis, but not the presence of arthritis/arthralgia.

Conclusion. The results suggest that anti-agalactosyl IgG antibody is frequently detected in SSc and is a serological indicator for more severe SSc. (J Rheumatol 2001;28:1847–51)

Key Indexing Terms:
SYSTEMIC SCLEROSIS ANTI-AGALACTOSYL IgG ANTIBODIES
PULMONARY FIBROSIS CONTRACTURE PHALANGES

Systemic sclerosis (SSc) is a multisystem disorder of connective tissue characterized by sclerotic changes in the skin and internal organs. Although the pathogenesis of this disease remains unknown, many immunologic abnormalities, including the presence of autoantibodies, have been detected, suggesting that SSc has an autoimmune background. SSc-specific autoantibodies, including anti-topoisomerase I antibodies, anticentromere antibodies, and anti-RNA polymerase antibodies, have been identified[1-3]. In addition, less specific autoantibodies such as rheumatoid factors (RF), anti-histone antibodies, and anti-single stranded DNA antibodies are also detected in SSc serum samples[4-6].

The Fc portion of IgG is the target of RF that are very frequently detected in patients with rheumatoid arthritis (RA). Although less frequently, RF are also positive in diseases other than RA including collagen diseases, liver injury, osteoarthritis, tuberculosis, sarcoidosis, and Crohn's disease[7]. In addition to the low specificity of RF to RA, the low sensitivity limits its clinical usefulness.

Anti-agalactosyl IgG antibodies (anti-AG IgG) were recently detected in sera from patients with RA[8]. Agalactosyl IgG is a glycoform of IgG found as a proportion of total IgG in all healthy individuals. Increased agalactosyl IgG levels have been described in sera from patients with RA[9-12]. RF in patients with RA have been shown to bind better to agalactosyl IgG than to galactosylated IgG. Anti-AG IgG activity therefore would be expected to have higher specificity and sensitivity than RF in RA[13]. Anti-AG IgG antibodies were detected in more than half of all seronegative patients with RA[14]. Further, the level of the antibody correlated positively with RA activity[15]. Thus anti-AG IgG may represent a useful marker for early RA and may be able to predict its course.

Anti-AG IgG have also been detected in some patients with collagen diseases including systemic lupus erythematosus (SLE) and Sjögren's syndrome[16,17]. We examined the prevalence and clinical correlation of anti-AG IgG in patients with SSc.

MATERIALS AND METHODS

Patients. Serum samples were obtained from 70 Japanese patients with SSc (66 women, 4 men). These patients were between 2 and 77 years old (mean 53). The mean duration of disease was 10 years (range 0–50). All patients fulfilled the criteria proposed by the American College of Rheumatology

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(ACR)[18]. Patients were grouped according to the classification system proposed by LeRoy, *et al*[19]: 49 patients (48 female, one male) had the limited form of SSc (lSSc) and 21 patients (18 female, 3 male) the diffuse form (dSSc). SSc patients with signs or symptoms suggestive of RA were excluded. Control serum samples were obtained from 20 age and sex matched healthy Japanese volunteers. Serum samples from 10 patients who fulfilled ACR criteria for RA[20] and 20 patients who fulfilled ACR criteria for SLE[21] were also examined. Fresh venous blood samples were centrifuged shortly after clot formation. All samples were stored at –70°C prior to use.

Clinical and laboratory data reported here were obtained at the time the serum samples were drawn. Patients had a detailed clinical assessment, and the involvement of their organ systems was investigated. Organ system involvement was defined as described by Steen, *et al*[22] with some modifications: lung = bibasilar fibrosis on chest radiograph; esophagus = hypomotility shown by barium radiography; joint = inflammatory polyarthralgias or arthritis; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment; and muscle = proximal muscle weakness and elevated serum creatine kinase. Erythrocyte sedimentation rates (ESR), C-reactive protein (CRP), IgG, IgA, and IgM were considered to be elevated when each value was higher than 20 mm/h, 0.5 mg/dl, 1774 mg/dl, 235 mg/dl, and 355 mg/dl, respectively. RF was measured by nephelometric method, and values > 20 IU/ml were considered positive.

Specificity of antinuclear antibody (ANA). ANA was detected by indirect immunofluorescence, using HEp-2 cells as the substrate, and by double immunodiffusion as described[23].

Measurement of anti-agalactosyl IgG antibodies. Anti-AG IgG in serum were measured with a lectin-enzyme immunoassay kit, the Eitest CARF (Eizai Co. Ltd, Tokyo, Japan) using human agalactosyl IgG as antigen[24]. The agalactosyl IgG was prepared from enzymatically treated oligosaccharides of human IgG. Human IgG was subsequently treated with neuraminidase in 0.1 M acetate buffer (pH 5.0) for 48 h at 37°C and beta-galactosidase in 0.1 M citrate-phosphate buffer (pH 7.0) for 24 h at 37°C. Agalactosyl IgG was purified using a protein G coupled to agarose as an affinity column for chromatography. The wells of polystyrene micro plates were coated with agalactosyl IgG. Standard solution or serum samples (100 μl) diluted to 1:201 were added. After 1 h incubation, the wells were washed and streptavidin-peroxidase conjugate solution (100 μl) was added. After a further 1 h of incubation and washing, chromogen substrate solution was added. The reaction was stopped with 2 mM sodium azide after 30 min, and the absorbance was read at 405 nm using a plate reader. The concentration of anti-AG IgG in serum samples was measured using a standard curve.

Statistical analysis. Statistical analysis was performed by Mann-Whitney U test for comparison of means, Fisher exact probability test for comparison of frequencies, and Bonferroni test for multiple comparisons. Spearman rank correlation coefficient was used to examine the relationship between 2 continuous variables. A p value < 0.05 was considered statistically significant. All data are shown as means ± SD.

RESULTS

Level and frequency of anti-agalactosyl IgG antibodies in SSc. Levels of anti-agalactosyl IgG in SSc patients (21.7 ± 27.6 AU/ml) were significantly higher than in controls (5.7 ± 3.1; $p < 0.05$) or patients with SLE (5.4 ± 9.5; $p < 0.05$), but lower than patients with RA (64.1 ± 57.4; $p < 0.001$; Figure 1). Anti-AG IgG levels in dSSc patients (34.8 ± 41.9) and lSSc patients (16.1 ± 16.1) were significantly elevated compared with controls ($p < 0.01$). Patients with dSSc had significantly increased levels of anti-AG IgG compared with lSSc ($p < 0.01$). Consistent with this, SSc patients with anti-topoisomerase I antibodies had significantly higher levels of anti-AG IgG than those with anticentromere antibodies (34 ± 41 vs 15 ± 15; $p < 0.01$; data not shown). Anti-AG IgG levels in patients with lSSc were significantly lower than patients with RA ($p < 0.001$), whereas there was no significant difference in antibody levels between RA and dSSc patients.

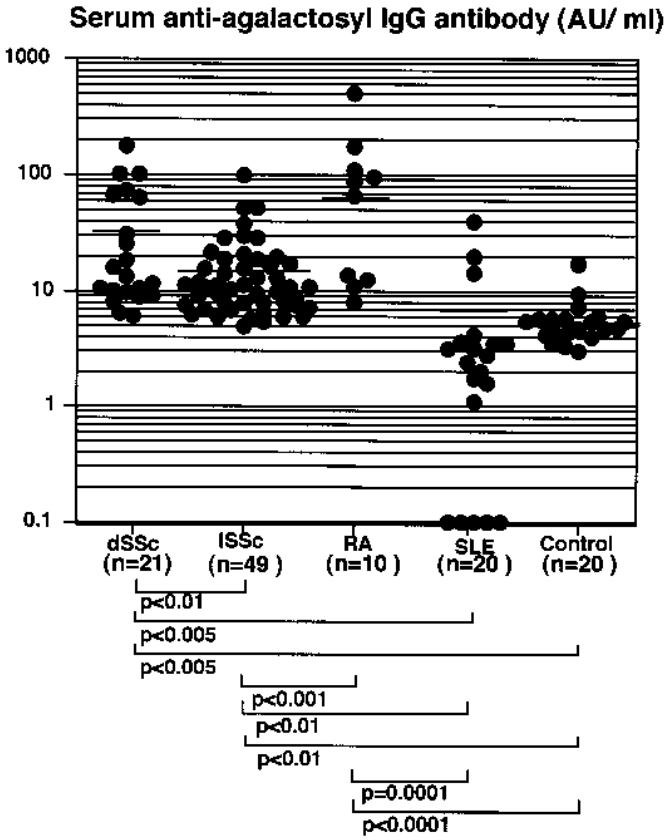

Figure 1. Anti-agalactosyl IgG antibody levels in patients with dSSc, lSSc, RA, and SLE, and healthy controls.

When the cutoff value of anti-AG IgG levels was set as 6 AU/ml[14], anti-AG IgG were detected in 52 (74%) of 70 patients with SSc, which was higher than the frequency of RF positivity in SSc (11/67, 16%). Anti-agalactosyl IgG antibody was positive in all (11/11) the patients with SSc with RF, while it was detected in 68% (38/56) of SSc patients with negative RF. Nine of 10 patients (90%) with RA had anti-AG IgG. Among 4 patients with seronegative RA, 3 had anti-AG IgG. Three of 20 controls were positive for anti-AG IgG. Thus, anti-AG IgG was much more frequently detected in patients with SSc than RF.

Clinical correlation of anti-agalactosyl IgG antibodies. SSc patients with pulmonary fibrosis showed significantly increased levels of anti-AG IgG compared to those without pulmonary fibrosis (42 ± 42 vs 14 ± 14; $p < 0.001$; Figure 2). Consistently, SSc patients with decreased %VC had significantly higher levels of anti-AG IgG than those with normal %VC (39 ± 43 vs 17 ± 19; $p < 0.01$). Further, anti-

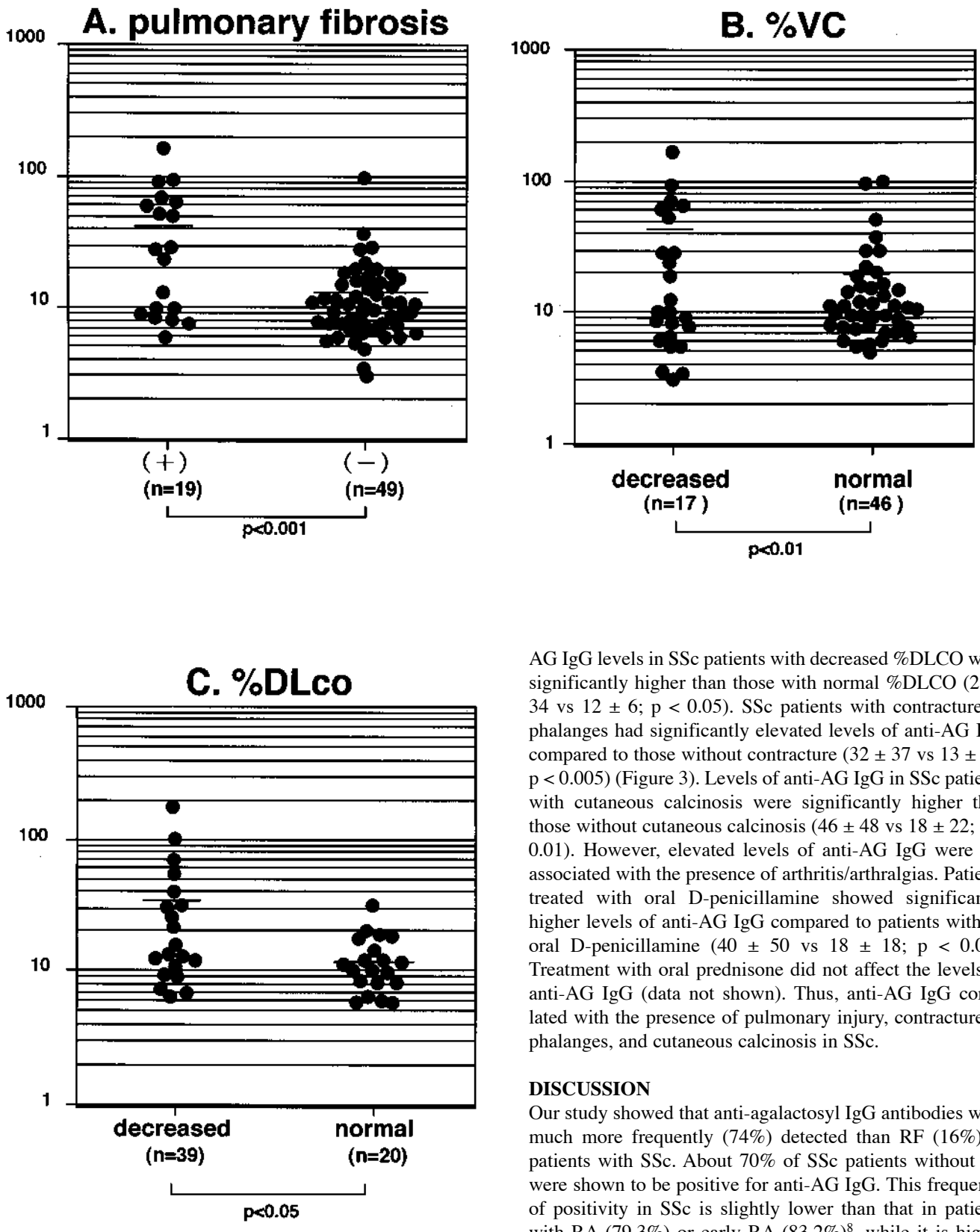

Figure 2. Anti-agalactosyl IgG antibody levels in SSc patients with or without pulmonary fibrosis, those with decreased or normal %VC, and those with decreased or normal %DlCO.

AG IgG levels in SSc patients with decreased %DLCO were significantly higher than those with normal %DLCO (28 ± 34 vs 12 ± 6; $p < 0.05$). SSc patients with contracture of phalanges had significantly elevated levels of anti-AG IgG compared to those without contracture (32 ± 37 vs 13 ± 10; $p < 0.005$) (Figure 3). Levels of anti-AG IgG in SSc patients with cutaneous calcinosis were significantly higher than those without cutaneous calcinosis (46 ± 48 vs 18 ± 22; $p < 0.01$). However, elevated levels of anti-AG IgG were not associated with the presence of arthritis/arthralgias. Patients treated with oral D-penicillamine showed significantly higher levels of anti-AG IgG compared to patients without oral D-penicillamine (40 ± 50 vs 18 ± 18; $p < 0.01$). Treatment with oral prednisone did not affect the levels of anti-AG IgG (data not shown). Thus, anti-AG IgG correlated with the presence of pulmonary injury, contracture of phalanges, and cutaneous calcinosis in SSc.

DISCUSSION

Our study showed that anti-agalactosyl IgG antibodies were much more frequently (74%) detected than RF (16%) in patients with SSc. About 70% of SSc patients without RF were shown to be positive for anti-AG IgG. This frequency of positivity in SSc is slightly lower than that in patients with RA (79.3%) or early RA (83.2%)[8], while it is higher than that in patients with SLE (32%) or Sjögren's syndrome (60%)[17]. Levels of anti-AG IgG in patients with SSc were

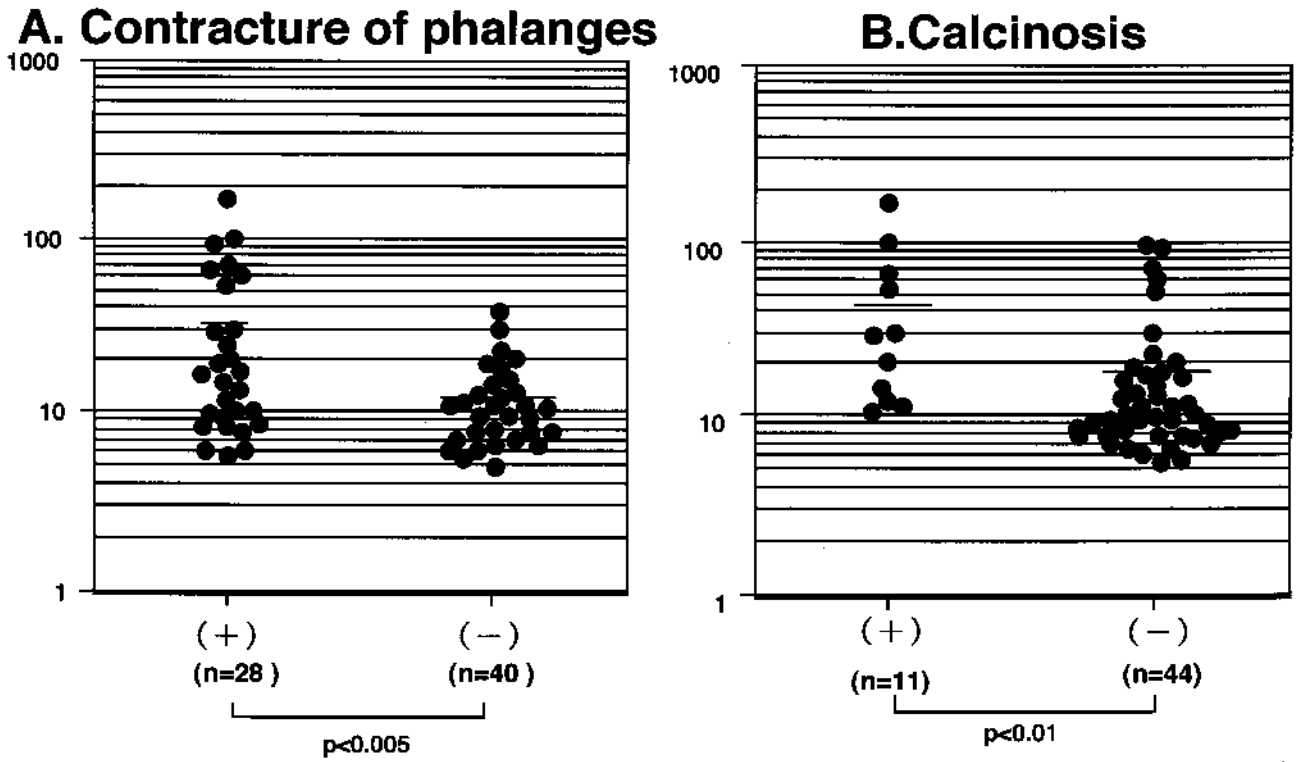

Figure 3. Anti-agalactosyl IgG levels in patients with or without contracture of phalanges and cutaneous calcinosis.

also significantly higher than controls, although they were lower than those in patients with RA. In patients with SSc, frequencies of SSc-specific autoantibodies, such as anti-topoisomerase I or anticentromere antibody, were 14–36%[25]. The frequency of anti-RNA polymerase antibodies and autoantibodies against nucleolar components was much lower[25]. The less specific autoantibodies, including anti-histone and anti-single stranded DNA antibodies, were present in 30–50% of SSc patients[6,26]. Although anti-AG IgG were not specific for SSc, the frequency of the positivity in SSc patients was highest among the autoantibodies detected in SSc patients. Concerning the clinical correlation, the elevated levels of anti-AG IgG were associated with more severe type of SSc, such as the diffuse form, or the presence of anti-topoisomerase I antibodies. This is consistent with the finding that levels of anti-AG IgG correlated with the presence or severity of pulmonary fibrosis. Thus the results suggest that anti-AG IgG are one of the autoantibodies frequently detected in SSc and are a serological marker for more severe type of SSc.

Articular involvement has been described as an initial manifestation in 12–65% of patients with SSc and as an eventual manifestation in up to 46–97%[27,28]. Brocka, *et al* have drawn attention to the rheumatoid-like changes in joint radiographs of a substantial minority of SSc patients, even in those patients specifically screened to exclude disease overlap with RA, suggesting that rheumatoid-like synovitis contributes to joint pathology in some patients[29]. Despite these findings, the synovial biopsies of SSc patients with more advanced disease consistently fail to show the expected progression typical of RA. A process of superficial fibrin accumulation and atrophy of the synovial lining cells occurs, which leads to a fibrosis similar to that observed in the overlying dermis[30,31]. This pathogenic process contributes to the development of contracture of phalanges. In this study, although anti-AG IgG were related to contracture of phalanges in SSc, anti-AG IgG levels were not associated with the presence of arthritis/arthralgia. Thus, although anti-agalactosyl IgG antibodies are related to joint involvement in both RA and SSc, anti-agalactosyl IgG antibody may be associated with contracture of phalanges in SSc.

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