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ORIGINAL ARTICLE

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# The levels of serum-soluble Fas in patients with rheumatoid arthritis and systemic sclerosis

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**Abstract** Different defects in Fas/APO-1 interaction with its ligand or in signaling of apoptosis may contribute to autoimmune disease. The aim of this study was to examine whether elevated serum-soluble Fas (sFas) levels are associated with rheumatoid arthritis (RA) or systemic sclerosis (SSc). sFas level was assayed using a sandwich ELISA in serum from 37 patients with RA, 30 patients with SSc and 20 healthy controls. The RA patients were classified according to disease activity, anatomical joint damage, and the presence of pulmonary involvement. Presence of pulmonary fibrosis, CO diffusion capacity (DLCO) and skin score were determined in patients with SSc. Serum sFas levels were not significantly different between study groups. Serum sFas level in the active RA patients was significantly higher than in the patients with inactive disease ($p < 0.05$). The untreated active RA patients had significantly higher sFas level than healthy controls ($p < 0.05$). In RA patients, sFas level was significantly correlated with rheumatoid factor titer ($p = 0.01$), C-reactive protein ($p < 0.05$), and erythrocyte sedimentation rate ($p < 0.05$). The RA patients with severe joint damage had significantly higher sFas level than those with mild joint damage ($p < 0.05$). The untreated SSc patients had significantly higher sFas levels than the treated SSc patients and healthy controls ($p < 0.01$). Serum sFas level was not correlated with presence of pulmonary fibrosis, DLCO or skin score. The soluble Fas molecule may provide a useful additional marker for assessment of disease activity and severity in patients with RA.

**Keywords** Apoptosis · Rheumatoid arthritis · Serum soluble Fas · Systemic sclerosis

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**Abbreviations** CRP: C-reactive protein · DLCO: CO diffusion capacity · ESR: Erythrocyte sedimentation rate · RA: Rheumatoid arthritis · RF: Rheumatoid factor · sFas: Serum-soluble Fas · SSc: Systemic sclerosis

## Introduction

Autoimmune rheumatic diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) or systemic sclerosis (SSc) have been associated with myriad immunologic abnormalities, ranging from increased proinflammatory cytokines, decreased anti-inflammatory cytokines, increased expression of major histocompatibility complex molecules, skewing of the T-cell receptor repertoire, increased numbers of B cells, and autoantibody production. It has been widely suspected that underlying all these immunological changes, there is a defect in the ability to eliminate self-reactive T cells or B cells resulting from a deficit in apoptosis [1]. Programmed cell death, or apoptosis, balances the cellular arm of the immune system. Appropriate regulation of the survival and deletion of immune cells allows necessary defense reactions and prevents self-damage. Apoptosis is therefore an essential mechanism to regulate the immune response and to maintain immunologic tolerance [2, 3].

Fas/APO-1/CD95 is a cell surface receptor belonging to the tumor necrosis factor-$\alpha$ receptor superfamily. Mutations in the Fas/APO-1 gene and Fas/APO-1 ligand gene have been shown to be the primary defect in the lpr and gld mouse models of autoimmunity [4, 5, 6]. The Fas molecule could occur as a soluble protein as well as a cell surface receptor. The soluble form of the Fas molecule (sFas), derived by alternative splicing from the same mRNA as the membrane form, seems to play an important role in signaling. It has been suggested that sFas modifies ligand concentration, downregulates membrane receptor numbers, and specifically inhibits ligand–receptor association in the extracellular space,

thus preventing the induction of apoptosis [7]. Recent studies have demonstrated an increase in serum sFas in patients with rheumatic diseases, especially SLE. It has been suggested that increased levels of sFas inhibit proper apoptosis of lymphocytes [7, 8, 9].

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation and progressive joint destruction. Synovial hyperplasia is typical in the inflamed joints of RA patients. Synovial cells have been thought to play a pivotal role in the pathogenesis of RA. Recent studies demonstrated that sFas is increased in synovium from patients with RA, and it was suggested that Fas-mediated apoptosis in RA synovial cells is involved early in the process of regression of hyperplastic synovial cells [10, 11, 12]. Hasunuma et al. [13] have also identified in the rheumatoid synovium infiltrating mononuclear cells that express functional Fas antigen. Even though several synoviocytes express the Fas antigen, most cells appear to escape the process of apoptosis, with the result that there is persistence of synovial hyperplasia in vivo. Therefore, Fas-mediated apoptosis is believed to be a useful tool for the treatment of RA.

The etiology and pathogenesis of SSc are still largely unknown. Whereas the advanced stages of SSc are characterized by the excessive accumulation of collagen, perivascular inflammation and endothelial cell apoptosis are already present in the early stages of the disease [14, 15]. Lymphocytes, and T cells in particular, seem to play an important role in the pathogenesis of SSc [16, 17]. The identification by a number of investigators of short- and long-lived fibroblast lines from SSc patients might be explained on the basis of defective apoptosis in the long-lived cell lines [18]. The possibility that SSc is at least partly based on an imbalance between fibroblast growth factors and fibroblast apoptosis is very appealing.

The aim of this study was to determine whether elevated serum sFas levels are associated with RA or SSc, and the indicators of disease activity and severity.

## Patients and methods

Thirty-seven patients with RA and 30 with SSc, recruited from a hospital-based sample, were included in the study. Demographic features of the patient groups are shown in Table 1. The patients fulfilled the respective classification criteria of the American College of Rheumatology [19, 20]. Twenty healthy subjects, 15 female and 5 male, were evaluated as the control group. Mean age was 50.4 ± 10.7 years in healthy subjects.

Serum sFas level in the study groups was assayed using a sandwich enzyme-linked immunosorbent assay (Quantikine, R&D Systems, Minneapolis, USA). Briefly, microplate wells were coated with a murine monoclonal antibody specific for Fas. After rinsing, microplate wells were blocked with 100 µl of phosphate-buffered sodium. Then, 100 µl of standard (recombinant human Fas/Fc chimera in a buffered protein base) or serum sample was added to each well and incubated for 2 h at room temperature. After washing, 200 µl of horseradish peroxidase-conjugated polyclonal antibody against Fas was added to each well and incubated for 2 h at room temperature, followed by another washing step. Then, 200 µl of substrate solution containing stabilized hydrogen peroxide and tetramethylbenzidine in a 1:1 ratio was added to each well and incubated for 30 min at room temperature. The reaction was stopped with 50 µl of 2 N sulfuric acid. The optical density of each well was read at 540–570 nm within 30 min. The detection limit of the assay was 20 pg/ml, with an intra-assay coefficient of variation of 4.6% and interassay coefficient of variation of 2.9%.

**Table 1** The demographic, clinical and laboratory features of the patients with RA and SSc

| | RA patients | SSc patients |
|---|---|---|
| Age (years) | 53.7 ± 13.5 | 46.2 ± 11.8 |
| Sex (female/male) | 27/10 | 27/3 |
| Median follow-up (months) | 42 (3–240) | 44 (2–210) |
| Pulmonary involvement (*n*/%) | 19/51.3 | 15/50.0 |
| Larsen's score | | |
| LS 1,2,3 (*n*/%) | 21/56.8 | |
| LS 4,5 (*n*/%) | 16/43.2 | |
| Rheumatoid factor titer (U/l) | 258 ± 454 | |
| Rheumatoid factor positivity (*n*/%) | 30/81.1 | |
| Serum C-reactive protein (mg/l) | 55 ± 51 | |
| Erythrocyte sedimentation rate (mm/h) | 62 ± 31 | |
| Hematocrit (%) | 35.8 ± 3.6 | |
| Skin score (points) | | 13.4 ± 7.7 |
| Diffusion capacity for carbon monoxide (%) | | 79 ± 28 |

The RA patients were classified according to disease activity, anatomical joint damage, and the presence of pulmonary involvement. Disease activity was assessed according to preliminary criteria for remission in RA from Pinals et al. [21]. Anatomical joint damage was classified using Larsen's score [22]. Rheumatoid factor (RF) titer, serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and hematocrit were determined in RA patients. Serum CRP and RF titers were measured by nephelometric immunoassay (Dade-Behring BN II, Liederbach, Germany), and values of more than 20 IU/ml in RF titer were considered positive. Hematocrit level was measured using a Coulter STKS device. Erythrocyte sedimentation rate was determined by the Westergren method.

The patients with SSc were classified according to the presence of pulmonary fibrosis. This was evaluated using high-resolution CT. Skin score and CO diffusion capacity (DLCO) were also determined in patients with SSc. Skin score (0 = normal skin; 3 = hidebound skin) was measured and summed for 15 anatomic areas by the method of Kahaleh et al. [23].

Results are given as mean ± standard deviation. Comparison of means was carried out using the Mann–Whitney *U* test. Correlations were analyzed using Spearman's correlation coefficient.

## Results

The clinical and laboratory findings of patients with RA or SSc are shown in Table 1. RF was positive in 30 patients with RA (81.1%). Twenty-eight patients with RA had active disease. Eighteen of these were on immunosuppressive therapy, including corticosteroids, methotrexate or azathioprine, and the remainder were receiving no medications. Nineteen RA patients (51.3%) had pulmonary involvement. The Larsen scores (LS) of RA patients were as follows: LS 1: 7 (18.9%), LS 2: 8 (21.6%), LS 3: 6 (16.2%), LS 4: 9, (24.3%), and LS 5: 7 patients (18.9%). The patients were classified according to LS as mild–moderate (LS 1, 2, and 3) and severe anatomical joint damage (LS 4, and 5). Fifteen patients with SSc (50%) had pulmonary fibrosis. Twelve SSc patients were receiving immunosuppressive drugs.

The mean serum sFas levels in the study groups are shown in Table 2. Serum sFas level in RA patients was not found to be significantly different from that in healthy subjects and SSc patients ($p > 0.05$). Serum sFas level in patients with active RA was significantly higher than those of the inactive patients ($p < 0.05$). Although the patients with active RA had a higher serum sFas levels than healthy subjects, the difference did not reach statistical significance ($p = 0.08$). The untreated active RA patients had significantly higher serum sFas levels than inactive RA patients ($p < 0.05$) and healthy subjects ($p < 0.05$), but the treated active RA patients did not ($p = 0.053$ and $p > 0.05$, respectively).

Serum sFas level was significantly higher in RA patients with severe joint damage (LS 4 and 5) than those of RA patients with mild or moderate joint damage ($9905 \pm 2014$ and $7911 \pm 3200$ pg/ml, respectively; $p < 0.05$). Serum sFas level was found to be similar among the RA patients with or without pulmonary involvement ($8270 \pm 3919$ and $8945 \pm 3142$ pg/ml, respectively, $p > 0.05$). There were significant correlations between serum sFas level and serum CRP ($r = 0.384$, $p < 0.05$), ESR ($r = 0.367$, $p < 0.05$), RF titer ($r = 426$, $p = 0.01$), and hematocrit ($r = -0.438$, $p < 0.01$).

Serum sFas level was similar between the SSc patients and healthy subjects ($p > 0.05$). It was significantly higher in untreated SSc patients than in treated SSc patients and healthy controls ($p < 0.01$ and $p < 0.01$, respectively). There was no significant difference in serum sFas level between the SSc patients with and without pulmonary fibrosis ($8790 \pm 2499$ and $8082 \pm 3990$ pg/ml, respectively; $p > 0.05$). It was also not significantly different between the untreated SSc patients with ($n = 8$) and without ($n = 10$) pulmonary fibrosis ($10417 \pm 2230$ and $10636 \pm 3821$ pg/ml, respectively; $p > 0.05$). Serum sFas level was not significantly correlated with DLCO in all patients ($r = -0.223$, $p > 0.05$) and untreated patients ($r = -0.255$, $p > 0.05$) with SSc. There was not a significant correlation between serum sFas level and skin score in patients with SSc ($r = -0.255$, $p > 0.05$).

**Table 2** Mean serum soluble Fas levels in the study groups

| Study groups | *n* | Serum sFas (pg/ml) |
|---|---|---|
| Rheumatoid arthritis | 37 | 8498 ± 4013 |
| Active RA | 28 | 9241 ± 3843[a] |
| Treated active RA | 18 | 9059 ± 4477 |
| Untreated active RA | 10 | 9569 ± 2499[a,b] |
| Inactive RA | 9 | 6186 ± 3832 |
| Systemic sclerosis | 30 | 8436 ± 3287 |
| Treated SSc | 12 | 6773 ± 2054 |
| Untreated SSc | 18 | 10527 ± 3007[c,d] |
| Healthy subjects | 20 | 7493 ± 3449 |

[a] $p < 0.05$ vs. inactive RA
[b] $p < 0.05$ vs. healthy subjects
[c] $p < 0.01$ vs. treated SSc
[d] $p < 0.01$ vs. healthy subjects

## Discussion

It has been reported that serum sFas levels were increased in autoimmune diseases and malignancies, and suggested that sFas may play a role in the pathogenesis of these diseases through downregulation of Fas-mediated apoptosis of activated lymphocytes and tumor cells. Cheng et al. [7] demonstrated that the serum sFas was increased in SLE patients, and they suggested that this increase in sFas leads to altered lymphocyte activation and proliferation. Nozawa et al. [9] and Christensson et al. [24] also found significantly increased levels of sFas in active SLE patients. As the high levels of sFas in lupus patients decreased following steroid therapy in study by Nozawa et al., these authors suggested that serum sFas level might also be an indicator of disease activity [9]. However, other studies in patients with SLE did not confirm these findings [25, 26, 27]. Because of the present study was done with a relatively small number of patients, no clear conclusions regarding the relationship between serum sFas levels and disease activity and severity in patients with RA or SSc may be drawn from it. However, this study provides some useful data about the role of sFas molecule in the assessment of activity and severity of RA.

The association between serum sFas level and RA or disease activity is controversial. Christensson et al. [24] reported that the serum sFas level is increased in RA patients; however, other authors [9, 12, 26] failed to show any differences in serum sFas levels between RA patients and healthy controls. In the present study, although sFas levels in patients with RA were similar to those in healthy subjects, the active RA patients had significantly higher serum sFas levels than in patients with inactive disease. In addition, we demonstrated that serum sFas level was significantly correlated with

markers of disease activity, such as serum CRP and ESR. We also observed a significant correlation between serum sFas level and RF titer. Utz and Anderson [28] showed that increased apoptotic cells contribute to autoantigen excess in SLE. Hasunuma et al. [12] reported that the synovial fluid concentration of sFas was higher in RA than in patients with osteoarthritis. They found significant correlations between the synovial fluid sFas level and serum CRP and ESR in RA patients. The serum concentration of sFas, however, was not different among the RA, osteoarthritis and control groups, and it was not correlated with disease activity indicators. Hashimoto et al. [29] shown that the sFas ligand concentration in the synovial fluid from RA patients with severe disease was significantly higher than that in synovial fluid from those with mild disease. In our study, serum sFas level was significantly higher in RA patients with severe joint damage (LS 4 and 5) than in RA patients with mild joint damage (LS 1–3).

In our study, serum sFas level in untreated active RA patients was significantly higher than in normal controls. Although serum sFas level was lower in active RA patients on immunosuppressive therapy than in untreated active patients, the difference did not reach statistical significance. It is known that the therapeutic effects of steroids, cyclophosphamide, azathioprine and methotrexate are potent inducers of apoptosis [1]. These findings suggest that immunosuppressive therapy may mediate apoptosis.

In the present study, serum sFas level was not found to be increased in patients with SSc. Wetzig et al. [30] and Stummvoll et al. [31] reported that the serum sFas levels in patients with SSc were significantly higher than those in healthy subjects. In other studies [9, 26, 32], however, no significant differences in serum sFas level were observed between patients with SSc and normal controls. The reason for these discordant results in serum sFas in SSc patients is unclear, but it relates either to differences in the assay systems or its performance, differences in disease activity, or differences in the use of treatment agents. In our study, the untreated SSc patients had significantly higher serum sFas levels than treated SSc patients and healthy subjects. This finding suggests that the active treatment for disease may affect serum sFas level.

In conclusion, we found higher levels of serum sFas in RA patients during active disease than in RA patients during inactive disease. Also, associations were observed between serum sFas level and measures of disease activity and severity in RA patients. These findings suggest that sFas molecule may have a clinical value for the assessment of disease activity and severity in RA patients, and also may play a role in the pathogenesis of RA. Immunosuppressive therapy may alter the apoptotic response in patients with RA or SSc. The intense focus by a number of investigators on the apoptosis process should lead to promising therapies directed at restoration of controlled apoptosis of abnormal cells that are associated with autoimmune diseases. Specific therapies that induce apoptosis without incurring side effects should improve the treatment of autoimmune disease.

## References


1. Mountz JD, Wu J, Cheng J, Zhou T (1994) Autoimmune disease: A problem of defective apoptosis. Arthritis Rheum 37: 1415–1420
2. Williams GT (1994) Apoptosis in the immune system. J Pathol 173: 1–4
3. Krammer PH, Behrmann I, Daniel P, Dhein J, Debatin KM (1994) Regulation of apoptosis in the immune system. Curr Opin Immunol 6: 279–289
4. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S (1992) Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 356: 314–317
5. Adachi M, Watanaba-Fukunaga R, Nagata S (1993) Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. Proc Natl Acad Sci USA 90: 1756–1761
6. Nagata S, Suda T (1995) Fas and Fas-ligand: lpr and gld mutations. Immunol Today 16: 39–43
7. Cheng J, Zhou T, Liu C et al. (1994) Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. Science 263: 1759–1762
8. Mysler E, Bini P, Drappa J et al. (1994) The apoptosis-1/Fas protein in human systemic lupus erythematosus. J Clin Invest 93: 1029–1034
9. Nozawa K, Kayagaki N, Tokano Y, Yagita H, Okumura K, Hasimoto H (1997) Soluble Fas (APO-1, CD95) and soluble Fas Ligand in rheumatic diseases. Arthritis Rheum 40: 1126–1129
10. Nakajima T, Aono H, Hasunuma T et al. (1995) Apoptosis and functional Fas antigen in rheumatoid arthritis synoviocytes. Arthritis Rheum 38: 485–491
11. Firestein G, Yeo M, Zvaifler N (1995) Apoptosis in rheumatoid arthritis synovium. J Clin Invest 96: 1631–1638
12. Hasunuma T, Kayagaki N, Asahara H et al. (1997) Accumulation of soluble Fas in inflamed joints of patients with rheumatoid arthritis. Arthritis Rheum 40: 80–86
13. Hasunuma T, Hoa TTM, Aono H et al. (1996) Induction of Fas-dependent apoptosis in synovial infiltrating cells in rheumatoid arthritis. Int Immunol 8: 1595–1602
14. Kahari VM (1993) Activation of dermal connective tissue in scleroderma. Ann Med 25: 511–518
15. Sgonc R, Gruschwitz MS, Dietrich H, Recheis H, Gershwin ME, Wick G (1996) Endothelial cell apoptosis is a primary pathogenic event underlying skin lesions in avian and human scleroderma. J Clin Invest 98: 785–792
16. Roumm AD, Whiteside TL, Medsger TA Jr, Rodnan GP (1984) Lymphocytes in the skin of patients with progressive systemic sclerosis. Quantification, subtyping, and clinical correlations. Arthritis Rheum 27: 645–653
17. Gustaffsson R, Tötterman TH, Klareskog L, Hallgren R (1990) Increase in activated T cells and reduction in suppressor inducer T cells in systemic sclerosis. Ann Rheum Dis 49: 40–49
18. Needleman BW, Ordonez JV, Taramelli D, Alms W, Gayer K, Choi J (1990) In vitro identification of a subpopulation of fibroblasts that produces high levels of collagen in scleroderma patients. Arthritis Rheum 33: 842–852
19. Arnett FC, Edworthy SM, Bloch DA et al. (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31: 315–324
20. Subcommittee for scleroderma criteria of the American Rheumatism Association diagnostic and therapeutic criteria committee (1980) Preliminary criteria for the classification of systemic sclerosis (Scleroderma). Arthritis Rheum 23: 581–590

21. Pinals RS, Masi AT, Larsen RA (1981) Preliminary criteria for clinical remission in rheumatoid arthritis. Arthritis Rheum 24: 1308–1315
22. Larsen A, Dale K, Eek M (1977) Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol Diagn 18: 481–491
23. Kahaleh MB, Sultany GI, Smith EA, Huffstutter JE, Loadholt B, LeRoy EC (1986) A modified scleroderma skin scoring method. Clin Exp Rheumatol 4: 367–369
24. Christensson M, Pettersson E, Eneslatt K et al. (2002) Serum sFas levels are elevated in ANCA-positive vasculitis compared with other autoimmune diseases. J Clin Immunol 22: 220–227
25. Knipping E, Krammer PH, Onel KB, Lehman TJA, Mysler E, Elkon KB (1995) Levels of soluble Fas/APO-1/CD95 in systemic lupus erythematosus and juvenile rheumatoid arthritis. Arthritis Rheum 38: 1735–1737
26. Goel N, Ulrich DT, Clair WS, Fleming JA, Lynch DH, Seldin MF (1995) Lack of correlation between serum soluble Fas/APO-1 levels and autoimmune disease. Arthritis Rheum 38: 1738–1743
27. Fujihara T, Takeuchi T, Tsubota K et al.(1998) Serum soluble Fas/APO-1 is increased in patients with primary Sjogren's syndrome. Clin Rheumatol 17: 496–499
28. Utz PJ, Anderson P (1998) Posttransplantation protein modifications, apoptosis, and the bypass of tolerance to autoantigens. Arthritis Rheum 41: 1152–1160
29. Hashimoto H, Tanaka M, Suda T et al. (1998) Soluble Fas ligand in the joints of patients with rheumatoid arthritis and osteoarthritis. Arthritis Rheum 41: 657–662
30. Wetzig T, Petri JB, Mittag M, Haustein UF (1998) Serum levels of soluble Fas/APO-1 receptor are increased in systemic sclerosis. Arch Dermatol Res 290: 187–190
31. Stummvoll GH, Aringer M, Smolen JS et al. (2000) Derangement of apoptosis-related lymphocyte homeostasis in systemic sclerosis. Rheumatology 39: 1341–1350
32. Tomokuni A, Aikoh T, Matsuki T et al.(1997) Elevated soluble Fas/APO-1 (CD95) levels in silicosis patients without clinical symptoms of autoimmune diseases or malignant tumours. Clin Exp Immunol 110: 303–309