

# Correlation Between Skin Fibrosis Based On Modified Rodnan Skin Score And B-Cell Activating Factor Serum In Systemic Sclerosis

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## ABSTRACT

**Background:** Progression and expansion of skin fibrosis are the most important characteristics in determining clinical responses and prognosis of Systemic Sclerosis (SSc). Using modified Rodnan skin score (mRSS) can not rapidly detect a slight changes of skin fibrosis in SSc patients. Biomarker assessment is needed to make a more objective, quantitative and rapid evaluation of the changes. Suggested potential useful biomarker is B-cell Activating Factor (BAFF), a positive regulator of B cell survival and maturation process. This study aimed to evaluate correlation between skin fibrosis based on mRSS and BAFF serum in SSc patients.

**Methods:** We used cross sectional methods. Enrolled all patients who met ACR EULAR 2013 criteria for SSc in Rheumatology Clinic Hasan Sadikin Hospital, Bandung, from November 2015 to March 2016. Subjects underwent medical record review, physical examination, mRSS measurement by rheumatologist, and blood tests. Data were analyzed using Rank-Spearman Correlation.

**Results:** Thirty seven subjects, with mean age  $40 \pm 10$  years old. Subjects consisted of 23(62.2%) limited SSc and 14(37.8%) diffuse SSc. Mean BAFF serum was  $1160.2 \pm 424.7$  pg/mL, no statistical difference were found between limited and diffuse type ( $p=0.662$ ). Median mRSS results was 16 ranged from 2 to 36. Correlation between mRSS and BAFF serum was not significant ( $r=0.077$ ;  $p=0.326$ ).

**Conclusion:** There is no correlation between mRSS and BAFF serum in systemic sclerosis at Hasan Sadikin Hospital.

**Keywords:** mRSS, BAFF, Systemic Sclerosis

## Introduction

Systemic sclerosis is a chronic, progressive autoimmune disease affecting multiple organs with unknown etiology. Systemic sclerosis affects patients' quality of life, psychology, physic, and economy.<sup>1</sup> The expansion and progression of tissue fibrosis is an important clinical response to predict prognosis of systemic sclerosis disease.<sup>2</sup> The Modified Rodnan Skin Score (mRSS) is the gold standard for evaluating skin fibrosis in systemic sclerosis patients.<sup>2,3</sup> Implementation of mRSS in daily practice is still a problem as it requires experience and repetitive teaching processes.<sup>4</sup> An

alternative marker or biomarker is needed to draw out the severity of skin fibrosis in more objective and rigorous way.<sup>2,5</sup> Further, the mentioned marker/biomarker should be highly correlated with the clinical manifestation used in mRSS scoring, so it can be used as an alternative test.<sup>6</sup>

Development in the knowledge regarding the pathophysiology of systemic sclerosis has found that B-cell plays roles in excess fibroblast activation and collagen production.<sup>7-10</sup> B-cell Activating Factor (BAFF) serves as a positive regulator of cell function by playing role in B-cell survival and maturation.<sup>8,11-13</sup> Researches regarding the correlation between mRSS and serum BAFF levels have been done previously, though there is several controversies regarding the results of those researches.<sup>10,14,15</sup> This study was expected to affirm the controversy that occurred in those previous studies by identifying the relationship between the degree of skin fibrosis based on mRSS and the level of serum BAFF in systemic sclerosis patients in our settings.

## Methodology

This is an analytic, descriptive research with a cross sectional design. Samples were collected consecutively at a specific time range. Subjects included in this study were all systemic sclerosis patients, who fulfill the ACR EULAR 2013 for SSc, came to Rheumatology Clinic of Hasan Sadikin Hospital, Bandung November 2015 till March 2016. Samples were excluded if they were known having cell malignancy (malignant lymphoma, multiple myeloma), overlap syndrome, mix connective tissue disease, and liver function disorders.

### Modified Rodnan Skin Score (mRSS) evaluation

mRSS were evaluated by a trained rheumatologist consultant. Skin thickening was assessed by palpation of 17 areas of body skin (fingers, hands, forearms, arms, feet, legs, thighs, face, chest, and abdomen) using scale of 0 (Zero) to 3. "0" for normal skin; "1" for mild thickness; "2" for moderate thickness; and "3" for severe thickness.

### B-cell Activating Factor (BAFF) test

Serum BAFF levels were measured using ELISA

sandwich technique. Blood serum were drawn on the same day of mRSS evaluation.

### Other Data

Other data were taken from patient's medical records, included: duration of illness; systemic sclerosis subtype; history of medication; and clinical manifestation based on ACR EULAR 2013. Additional laboratory analysis conducted were complete blood counts, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), creatinine, and urinalysis.

### Statistical analysis

Data normality was tested using the Shapiro-Wilk test, and bivariate analysis was done using the Rank Spearman Correlation Test.

### Results

There were 37 subjects recruited in this study, with an average age of  $40 \pm 10$  years. The youngest patient was 16 years old and the oldest were 62 years old. Subjects were divided into groups based on SSc type – 14 subjects (37.8%) had diffuse systemic sclerosis and 23 subjects (62.2%) had limited systemic sclerosis. Four subjects (10.8%) included were diagnosed with systemic sclerosis for the first time, and had never received DMARD. The median of illness duration was 36 months with ranged from 3 months to 17 years. Basic characteristics of the subjects were served in Table 1.

**Table 1.** Basic Characteristics

Characteristics (units)	Results
<b>Age</b>	$40 \pm 10$ years*
<b>Gender, N (%)</b>	
Male	1 (2.7)
Female	36 (97.3)
<b>Systemic sclerosis type, N (%)</b>	
Limited	23 (62.2)
Diffuse	14 (37.8)
<b>Patient Type, N (%)</b>	
Old	33 (89.2)
New	4 (10.8)
<b>Illness Duration</b>	36 (3 - 204) months**
<b>Medication History, N (%)</b>	
Methotrexate	33 (89.2)
Steroids	29 (78.4)
Cyclophosphamide	2 (5.4)
Diltiazem	7 (18.9)
Nifedipine/Amlodipine	22 (59.5)
Aspilet	17 (45.9)
<b>ACR EULAR 2013 Clinical Manifestation, N (%)</b>	
Finger Fibrosis	37 (100)
Finger Edema	14 (37.8)
Finger Skar	28 (75.7)
Telangectasia	12 (32.4)
Salt and Pepper Appearance	20 (54.0)
Raynaud's Phenomenon	29 (78.4)
<b>mRSS</b>	16 (2 - 36)**

\*: average  $\pm$  standard deviation (normal distribution data); \*\*: median (min-max), for not normal distribution data.

The average serum BAFF found in this research was  $1160.2 \pm 424.7$  pg/mL. The average serum BAFF level in the limited-type systemic sclerosis group was  $1132.8 \pm 470.5$  pg/mL,

while in the diffuse-type systemic sclerosis group was  $1205.2 \pm 348.5$  pg/mL. There was no significant difference in the serum BAFF levels between two groups, as shown in table 3. The median mRSS among the subjects was 16, the lowest level was 2 and the highest 36. There was a significant difference in the mRSS levels of patients with diffuse and limited-type of systemic sclerosis, with  $p < 0.001$ , as shown in table 3.

**Table 2.** Laboratory Result

Characteristics (units)	Results
<b>Laboratory</b>	
Hb (g/dL)	$13.2 \pm 1.2^*$
Leucocyte (/mm <sup>3</sup> )	$9932 \pm 3176^*$
Thrombocytes (/mm <sup>3</sup> )	$314.703 \pm 86.570^*$
LED (mm/jam)	28 (1 - 87)**
SGOT (U/L)	17 (11 - 59)**
SGPT (U/L)	13 (5 - 42)**
Creatinine (mg/dL)	0.67 (0.44 - 1.92)**
Proteinuria (n (%))	
Neg	31 (83.8 %)
1+	6 (16.2 %)
<b>BAFF (pg/mL)</b>	$1160.2 \pm 424.7^*$

\*: average  $\pm$  standard deviation (normal distribution data); \*\*: median (min-max), for not normal distribution data.

**Table 3.** Analysis of the BAFF and mRSS difference between the Limited and Diffuse Types

Variable	Limited Type n=23	Diffuse Type n=14	p-value
<b>BAFF (pg/mL)</b>	$1132.8 \pm 470.5^*$	$1205.2 \pm 348.5^*$	0.622
<b>mRSS</b>	12 (2-27)**	27(14-36)**	<0.001

Note: T-test analysis, significant if p-value<0.05

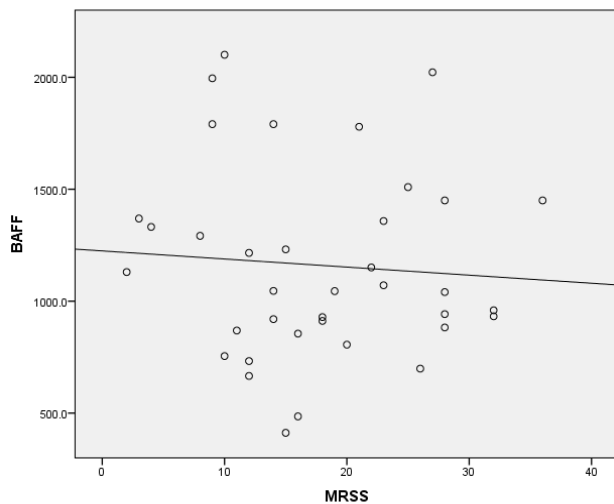
\*: average  $\pm$  standard deviation (normal distribution data); \*\*: median (min-max), for not normal distribution data.

Bivariate analysis of the severity of skin fibrosis based on the mRSS and serum BAFF levels showed no significant correlation between mRSS and serum BAFF levels ( $r = -0.077$ ,  $p = 0.326$ ), as shown in table 4. Bivariate analysis on the newly diagnosed patients, or those who have never received DMARDs, showed that there is a positive correlation between mRSS and the serum BAFF levels, though there was no significant statistical difference ( $r = 0.400$ ,  $p = 0.300$ ), as shown in table 5. Analysis subjects based on history of using steroid also showed no significant correlation between mRSS and the BAFF levels in patients who have used and have never used steroids, as shown in table 5.

**Table 4.** mRSS and BAFF Serum Bivariate Analysis

Variable	mRSS	
	R	p-value
BAFF (pg/dL)	-0.077	0.326*

Note: Rank Spearman correlation analysis, \*significant if  $p < 0.05$



**Figure 1. mRSS and Serum BAFF Distribution Diagram**

**Table 5. mRSS and BAFF Bivariate Analysis Based on Drug Type**

Variable	Group	mRSS	
		R	p-value
BAFF (pg/mL)	Receiving DMARD (n=33)	-0.122	0.250
	Not receiving DMARD (n=4)	0.400	0.300
	Receiving Steroid (n=29)	-0.013	0.473
	Not receiving steroid (n=8)	-0.524	0.091

Note: Rank Spearman Correlation Analysis, significant if p-value < 0,05

## Discussion

Average age of the subjects was  $40 \pm 10$  years. It is consistent with the literature which stated most systemic sclerosis occur in the third and fourth decades of life.<sup>16,17</sup> The average age of our subjects do not differ greatly from the previous research conducted by Fawzy, et al, which reported average age of  $43.75 \pm 14$  years<sup>14</sup>, and Abdo, et al research, which reported average age of  $38.2 \pm 12.1$  years.<sup>15</sup> Subjects were dominated by women, account for 97.3% of all subjects. This is consistent with the fact that systemic sclerosis mostly occurs in women.<sup>16,18</sup>

We found Raynaud's phenomenon in 29 subjects (78.4%). Raynaud's phenomenon is the most common complaint at the beginning of the illness.<sup>18,19</sup> Research conducted by Abdo, et al showed only 13 (21.7%) subjects suffered from Raynaud's phenomenon, whereas Fawzy, et al found that all subjects (12 patients) with Raynaud's phenomenon.<sup>14,15</sup> Differences of this clinical manifestations may be attributed with the management and the onset of the disease. The high percentage of Raynaud's phenomenon as reported by Fawzy, et al may be occurred because all recruited subjects had not received DMARD or corticosteroid therapy. While, in our research as well as in Abdo, et al study, major subjects had received DMARDs and other symptomatic therapy routinely.

The average BAFF level in this research was  $1160.2 \pm 424.7$  pg/mL. Fawzy reported a similar BAFF level of  $1060 \pm 290$  pg/mL, whereas Abdo, et al also reported a relative similar BAFF level of  $1100 \pm 835.4$  pg/mL<sup>14,15</sup> In this research, we found no significant difference in the BAFF levels of patients with diffuse and limited systemic sclerosis ( $p = 0.622$ ), as shown in table 4. A similar result was reported by Abdo, et al

( $p = 0.370$ ),<sup>15</sup> but it is not concordance with the result reported by Matsushita, et al and Fawzy, et al.

Various factors may influence the results of the correlation between BAFF level and MRSS result. We suggested the difference result between our research and Abdo, et al study, compared to the research by Matsushita, et al and Fawzy, et al were happened due to the medication received by the subjects. In this research, 89.2% subjects had already received methotrexate. Only 4 patients who were newly diagnosed with systemic sclerosis had never received methotrexate. Steroids had also been given to 29 subjects (78.4%), and two subjects (5.4%) were undergoing cyclophosphamide chemotherapy. The medication characteristics in this study was quite similar to the research conducted by Abdo, et al where 65% patients were receiving methotrexate and oral corticosteroids, and 21.7% patients were undergoing cyclophosphamide chemotherapy.<sup>15</sup>

This subjects' medication characteristic was differed greatly from the study conducted by Matsushita, et al. which from the 83 patients enrolled, only 5 were receiving low dose corticosteroids, and 8 were receiving low dose D-penicillamine, while the others were not received any other immunosuppressive therapy.<sup>10</sup> Likewise the medication characteristic in research conducted by Fawzy, et al, All subjects were a newly diagnosed SSc patients, so they had never received corticosteroids or DMARD therapy before the study began.<sup>14</sup>

Methotrexate is a conventional DMARD. It is an anti-metabolite drug which inhibits the dihydrofolate reductase enzyme and causes a disturbance in the formation of DNA and nucleotides.<sup>20</sup> Low dose methotrexate can be used to treat inflammatory of an autoimmune disease. The effects of methotrexate on the immune system include decreasing proinflammatory cytokines, releasing extracellular adenosine, and inhibiting activation of T-cell.<sup>20</sup>

Methotrexate is the chosen therapy for early phase skin fibrosis in diffuse systemic sclerosis.<sup>20,21</sup> The efficacy of methotrexate towards skin fibrosis has been studied in two randomized controlled trials but no significant difference were reported in those trials. Van den Hoogen, et al study, reported higher mRSS improvement after receiving 15 mg intramuscular methotrexate for 24 weeks in comparison to placebo ( $p = 0.06$ ).<sup>22</sup> Pope, et al study, also reported an improvement of mRSS after 12 months methotrexate treatment, though no significant difference were found as the number of subjects were too small.<sup>23</sup>

Methotrexate can influence the number and the activity of B and T cells, and possibly the level of BAFF in the blood. Therefore, we suggested that subjects who have received DMARDs, specifically methotrexate, will not showing any correlation between mRSS and serum BAFF level. This is supported by individual analysis of the correlation between mRSS and BAFF in 4 naïve subjects included in our study, where a moderate correlation was identified ( $r=0.400$ ;  $p=0.300$ ), though not statistically significant was found as the sample size is relatively small. Therefore, the use of DMARD is yet to be determined as a factor that influences the level of BAFF.

Other suggestion of medication that can influence the insignificant correlation result is the use of corticosteroid. Corticosteroids are the most common drug used for systemic sclerosis<sup>24</sup>, although it has never undergone any trials regarding its efficacy and safety. Even, at high dose, corticosteroid was reported have correlation with the occurrence of renal crisis.<sup>24</sup> The use of steroids in autoimmune diseases can cause transient lymphocytopenia by changing the way lymphocytes re-circulate, and also inducing lymphocyte death. The primary immune suppression effects of steroids is the inhibition of cytokines and T cell activation.<sup>20</sup> With those direct effects on lymphocytes, the use of corticosteroids may have an effect on the serum BAFF level too. However, researches regarding the effect of corticosteroids on BAFF levels have never been done, and the presence of a direct effect of steroids on BAFF is still unknown. In this study, we also analyzed the correlation between mRSS and the BAFF level in patients using and not using steroids, and no significant correlation was found with either groups (Table 5).

We realize some limitations on this study that may affect the study results. Most subjects have received DMARD therapy, that can affect the number of B cells and T cells, and we suggested might also affect the level of BAFF in the serum. Besides, duration and DMARD dose may also affect our observation result. However, we only recorded the type of DMARD drugs, but not recorded the duration of treatment and dosage of the drug.

### Conclusion

In conclusion, we found no significant correlation between mRSS and the level of BAFF in SSc patients. The BAFF level might be affected by medication received by the patients, especially methotrexate. Patients undergo DMARD therapy showed no correlation between mRSS and BAFF, whereas naïve patients might have a moderate correlation between mRSS and BAFF. To provide better understanding of pathogenesis and relationship between mRSS and BAFF, there should be larger-scale studies addressing naïve subject, with cohort or case control design studies.

### Conflict of Interest

All author reported no conflict of interest regarding the study.

### References

- Bernatsky S, Hudson M, Panopalis P, Clarke AE, Pope J, Leclercq S, et al. The cost of systemic sclerosis. *Arthritis Care & Research*. 2009;61(1):119-23.
- Castro SV, Jimenez SA. Biomarkers in systemic sclerosis. *Biomarkers in medicine*. 2010;4(1):133-47.
- Czirjak L, Foeldvari I, Muller-Ladner U. Skin involvement in systemic sclerosis. *Rheumatology (Oxford, England)*. 2008 Oct;47 Suppl 5:v44-5.
- Czirjak L, Nagy Z, Aringer M, Riemekasten G, Matucci-Cerinic M, Furst DE. The EUSTAR model for teaching and implementing the modified Rodnan skin score in systemic sclerosis. *Annals of the rheumatic diseases*. 2007 Jul;66(7):966-9.
- Moinzadeh P, Denton C, Abraham D, Ong V, Hunzelmann N, Eckes B, et al. Biomarkers for skin involvement and fibrotic activity in scleroderma. *Journal of the European Academy of Dermatology and Venereology*. 2012;26(3):267-76.
- Lafyatis R. Application of Biomarkers to Clinical Trials in Systemic Sclerosis. *Current Rheumatology Reports*. 2012;14(1):47-55.
- Mariette X. How does BAFF activate B cells in patients with autoimmune diseases.
- Bosello S, Pers J, Rochas C, Devauchelle V, De Santis M, Daridon C, et al. BAFF and rheumatic autoimmune disorders: implications for disease management and therapy. *International journal of immunopathology and pharmacology*. 2007;20(1):1.
- François A, Chatelus E, Wachsmann D, Sibilia J, Bahram S, Alsaleh G, et al. B lymphocytes and B-cell activating factor promote collagen and profibrotic markers expression by dermal fibroblasts in systemic sclerosis. *Arthritis Res Ther*. 2013;15:R168.
- Matsushita T, Hasegawa M, Yanaba K, Koderia M, Takehara K, Sato S. Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis & Rheumatism*. 2006;54(1):192-201.
- Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. *Nature Reviews Immunology*. 2002;2(7):465-75.
- Ng LG, Sutherland AP, Newton R, Qian F, Cachero TG, Scott ML, et al. B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells. *Journal of immunology (Baltimore, Md : 1950)*. 2004 Jul 15;173(2):807-17.
- Krivosikova M, Dallos T, Maslinski W, Buc M. B cell activating factor, its role in autoimmunity, and targeting in autoimmune diseases. *Bratislavské lekárske listy*. 2008;110(3):137-45.
- Fawzy SM, Gheita TA, El-Nabarawy E, El-Demellawy HH, Shaker OG. Serum BAFF level and its correlations with various disease parameters in patients with systemic sclerosis and systemic lupus erythematosus. *The Egyptian Rheumatologist*. 2011 1//;33(1):45-51.
- Abdo MS, Mohammed RHA, Raslan HM, Gaber SM. Serum B-cell activating factor assessment in a population of Egyptian patients with systemic sclerosis. *International Journal of Rheumatic Diseases*. 2013;16(2):148-56.
- Varga J. Systemic sclerosis: epidemiology, pathology and pathogenesis. *Primer on rheumatic diseases 13th ed Atlanta: Springer*. 2008:351-8.
- Denton C, Black C, Korn J, De Crombrughe B. Systemic sclerosis: current pathogenetic concepts and future prospects for targeted therapy. *The Lancet*. 1996;347(9013):1453-8.
- Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *New England Journal of Medicine*. 2009;360(19):1989-2003.
- Mayes MD. Systemic sclerosis. *Primer on the rheumatic diseases*. Springer; 2008. hlm. 343-62.
- Manno R, Boin F. Immunotherapy of systemic sclerosis. *Immunotherapy*. 2010 Nov;2(6):863-78.
- Kowal-Bielecka O, Landewe R, Avouac J, Chwiesko S, Miniati I, Czirjak L, et al. EULAR recommendations for the treatment of systemic sclerosis: a report from the EULAR Scleroderma Trials and Research group (EUSTAR). *Annals of the rheumatic diseases*. 2009;68(5):620-8.
- Van den Hoogen F, Boerbooms A, Swaak A, Rasker J, Van Lier H, Van De Putte L. Comparison of methotrexate with placebo in the treatment of systemic sclerosis: a 24 week randomized double-blind trial, followed by a 24 week observational trial. *Rheumatology*. 1996;35(4):364-72.
- Pope JE, Bellamy N, Seibold JR, Baron M, Ellman M, Carette S, et al. A randomized, controlled trial of methotrexate versus placebo in early diffuse scleroderma. *Arthritis & Rheumatism*. 2001;44(6):1351-8.
- Vitiello M, Abuchar A, Santana N, Dehesa L, Kerdel FA. An Update on the Treatment of the Cutaneous Manifestations of Systemic Sclerosis: The Dermatologist's Point of View. *Journal of Clinical & Aesthetic Dermatology*. 2012;5(7).