

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/359435707>

Difference between the Decrease in P1NP Concentration and MRSS Within 3 Months Treatment of Systemic Sclerosis

Article · March 2022

DOI: 10.37275/IJR.v13i2.184

CITATIONS

0

READS

19

3 authors:



Devy Priyantini Hanafi

1 PUBLICATION 0 CITATIONS

SEE PROFILE



Sumartini - Dewi

Padjajaran University

90 PUBLICATIONS 140 CITATIONS

SEE PROFILE



Andri Reza Rahmadi

Padjajaran University

51 PUBLICATIONS 290 CITATIONS

SEE PROFILE



Difference between the Decrease in P1NP Concentration and MRSS Within 3 Months Treatment of Systemic Sclerosis

Devy Priyantini Hanafi^{1*}, Sumartini Dewi², Andri Reza Rahmadi²

¹ Department of Internal Medicine, Faculty of Medicine, Universitas Padjadjaran/ Hasan Sadikin General Hospital, Bandung, Indonesia

² Division of Rheumatology, Faculty of Medicine, Universitas Padjadjaran/ Hasan Sadikin General Hospital, Bandung, Indonesia

ARTICLE INFO

Keywords:

Fibrosis
MRSS
P1NP
Systemic Sclerosis
Therapeutic Response

Corresponding author:

Devy Priyantini Hanafi

E-mail address:

dr.devypriyantini@gmail.com

All authors have reviewed and approved the final version of the manuscript.

<https://doi.org/10.37275/IJR.v13i2.184>

ABSTRACT

Introduction: Systemic sclerosis is characterized by extensive and progressive organ fibrosis leads to organ failure and death. Progression of skin thickening is a predictor of morbidity and mortality. Due to the limitation of modified Rodnan skin score (MRSS) sensitivity in detecting small changes in skin fibrosis, thus we proposed Procollagen Type I N-Terminal Propeptide (P1NP) as potential biomarker. This study aimed to analyze the difference between the decrease in P1NP concentration and MRSS within 3 months treatment of systemic sclerosis.

Methods: We conducted a retrospective cohort of paired numerical comparative analytic methods, as follow up of a study done by Vincent et al. and Dewi et al. Analisis of MRSS changes and serum P1NP concentrations were done prior to treatment (baseline), and on week 4th, 8th and 12th treatment. **Result:** Fifty-nine subjects were enrolled in the study. We analyzed the results of P1NP and MRSS at 4th, 8th and 12th weeks of treatment, there was a significant decrease in mean rank of P1NP and MRSS ($p=0.033$ and <0.001). The effect of MRSS change was greater than P1NP. The highest decreasing effect of MRSS was obtained at week 8th ($\eta^2 = 0.424$, 42.4% decrease effect), and the largest decrease effect of P1NP was obtained at week 12th ($\eta^2 = 0.120$; 12% decrease effect). **Conclusion:** There was a difference in decreasing P1NP concentrations and MRSS in systemic sclerosis within 3 months of observation. MRSS showed a larger decrease in change than P1NP after treatment.

1. Introduction

Systemic sclerosis is an autoimmune disease with a complex pathophysiology and heterogenous clinical manifestation involving many organs. The etiology of systemic sclerosis is still unknown certainly. The severity of systemic sclerosis and prognosis differ in each patients depending on organ involvement.¹ The clinical course of systemic sclerosis is difficult to predict, organ fibrosis can be widespread and

progressive caused organ failure and death, the major caused of morbidity and mortality this disease.^{2,3}

Modified Rodnan skin score (MRSS) is a measuring tool for assessing skin fibrotic disorder or skin sclerosis, that has been proven in many clinical trials.^{1,4} A study correlated MRSS with skin biopsy results as the gold standard for assessing the degree

of skin fibrosis.⁵ The progression of skin thickening can be a predictor of mortality and organ involvement,⁶ but the interclass correlation coefficient on the MRSS is low when performed by inexperienced rheumatologist.⁷ MRSS examination assess the new changes after 3 to 6 months of therapy.⁸⁻¹⁰ MRSS may not very sensitive to smaller changes, but important for skin thickening, therefore needs other markers those more specific and accurate for skin fibrosis assessment.¹¹

A potential biologic marker of systemic sclerosis to assess fibrosis progression is P1NP, which is the result of the degradation of collagen synthesis in the fibrosis process.¹² The aim of this study is to find a relationship between P1NP and MRSS. The similar studies had been done before, but results were still controversial. The controversy might be influenced by race and ethnicity.¹³⁻¹⁵ A study to determine the relationship between procollagen type 1 N-terminal propeptide serum levels and MRSS in patients with systemic sclerosis had been carried out at the Rheumatology clinic and inpatient department of Internal Medicine, Dr. Hasan Sadikin General Hospital Bandung from May 2016 to July 2016 by Vincent et.al.¹⁶ There is a significant correlation between P1NP and MRSS with a moderate degree of significance. This study is a follow-up study that followed Vincent's research subjects for 3 months of treatment. The examination was done every month, to determine difference in the decrease in serum P1NP concentration with systemic sclerosis disease activity based on MRSS followed in 3 months of treatment.

2. Methods

Subject

Systemic sclerosis outpatient dr. Hasan Sadikin General Hospital Bandung who met the 2013 ACR/EULAR criteria and followed the research of Vincent et. al. and Dewi S et al.¹⁷ from May 2015 to June 2017. This study used secondary data from previous study of Vincent et. al. and Dewi S et al.

Subjects were excluded if there was comorbid osteoporosis, liver cirrhosis, metastatic bone malignancy, postmenopausal women, have chronic kidney failure, and did not follow the study for 3 months.

MRSS examination

The degree of skin fibrosis based on MRSS was assessed by a consultant rheumatology of Internal Medicine Department, Faculty of Medicine Universitas Padjadjaran/RSHS. The examiner has a good intraclass correlation coefficient (>0.7) in the MRSS examination among the rheumatology consultants on the Interobserver variability agreement test.

MRSS examination is an assessment of disease activity based on the degree of skin fibrosis which is assessed in 17 areas measured with a score range of 0-3, score of 0 describes normal skin; 1 describes mild/minimal crusting; 2 describes moderate crusting without deeper tissue fixation; 3 represents severe crusting and is fixed to the underlying tissue. MRSS was assessed monthly for 3 months of treatment by 1 rheumatology consultant.

P1NP serum levels

Examination of P1NP serum levels continuously tested along with MRSS examination, using the sandwich ELISA method with units of ng/mL. Serum P1NP levels were tested monthly for 3 months of treatment.

Type of treatment

The types of treatment given to the subjects of this study were methotrexate DMARD, mycophenolate mofetil, sulfasalasin, steroids, aspirin, clopidogrel, cyclophosphamide chemotherapy, calcium channel blockers, and ciplukan herbal therapy. The type of drug given was not homogeneous, it was given according to the patient's needs, and some subjects received ciplukan herbal therapy, which given during Dewi's study.

During the 3 months of observation there was no change in the type of drug in each patient, each patients, they received same type of medications every month.

Statistical analysis

This study is a retrospective cohort study using statistical methods with paired numerical comparative analysis. Statistical calculations are as follows: The description of characteristics of the research data is presented with the number and percentage. The MRSS variable is transformed from ordinal to numeric with a scale of 100. Hypothesis using a comparative analysis of paired numerical data based on the results of repeated measurements of more than two measurements with one way ANOVA repeated measure, then calculated eta square to see the effect of the decrease in P1NP and MRSS changes. Data processed using SPSS Ver.20 software with a significance value of $p < 0.05$.

3. Results

This study used secondary data "Double-blind Clinical Trial of Ciplukan Herb Extract on Clinical Improvement of Skin Disorders, Inflammatory Processes, Immunology and Fibrosis in Scleroderma Patients",¹⁷ from May 2015 to June 2017.

Subjects of study were fifty-nine patients who had met the inclusion and exclusion criteria and had complete P1NP laboratory examination data from sixty-one available research subject data.

Description of the characteristic subjects was presented in Table 1. Based on the characteristics, most of the subjects were women (96.6%) with a mean age 42 years, with a range between 21 to 57 years. Subjects were divided; diffuse type systemic sclerosis were 35 (59.3%) subjects and limited type 24 (40.7%) subjects. Duration of systemic sclerosis was less than two years in 18 (30.5%) subjects and

more than two years in 41 (69.5%) subjects. The median length of illness of the subjects was 26 months, with a range from 6 months to 8 years.

The treatment types given to the subjects of this study was DMARD methotrexate to 56 (94.9%) subjects, mycophenolate mofetil to 3 (0.33%) subjects, sulfasalazin to 1 (0.16%) subjects, steroids to 31 (52.5%) subjects. The number of subjects who received aspirin and clopidogrel therapy were 46 (77.9%) subjects and 7 (11.8%) subjects, respectively. Three (5.1%) subjects were undergoing cyclophosphamide chemotherapy due to pulmonary involvement and calcium channel blockers were given to 59 (100%) subjects due to complaints of Raynaud's phenomenon. In this study, there were 29 (49.2%) subjects who received ciplukan herbal therapy, which was given during Dewi's study.¹⁷

The most clinical symptoms based on the 2013 ACR EULAR diagnostic criteria were finger fibrosis 59 (100%) subjects and Raynaud's phenomenon 59 (100%) subjects, scar or ulcer on the finger 36 (61%) subjects, finger edema 54 (91.5%) subjects, salt and pepper appearance 32 (54%) subjects, and teleangiectasia 17 (28.8%) subjects.

The average routine hematological parameters in this study were within normal limit. The average hemoglobin level was 12.7 g/dL with a range of 10.5-15.1 g/dL, the median leukocyte was 7,950 (4,490-19,400/mm³), and the mean platelet was 335,017 ± 90,136/mm³. The median value of LED was 38 mm/hr with a range from 16 mm/hr to 60 mm/hr. All subjects were screened against the exclusion criteria for this study, liver cirrhosis, post menopause, malignancies that metastasize to bone, chronic kidney disease, and osteoporosis.

Table 1. Characteristic of study subjects

Characteristic (units)	n (%)	Median (min-max) or Mean ± SB
Age (year)		42 (21 – 57)
Sex		
Male	2 (3,4)	
Female	57 (96,6)	
Female : Male	28 : 1	
Systemic type of sclerosis		
Limited	24 (40,7)	
Diffused	35 (59,3)	
Time of suffering systemic sclerosis		
≤2 years	18 (30,5)	
>2 years	41 (69,5)	
Disease duration (months)		26 (6 - 96)
History of treatment		
Methotrexate	56 (94,9)	
Mycophenolate mofetil	2 (0,33)	
Sulfasalazine	1 (0,16)	
Steroids (methylprednisolon)	31 (52,5)	
Calcium channel blocker	59 (100)	
Aspilet	46 (77,9)	
Clopidogrel	7 (11,8)	
Cyclophosphamide	3 (5,1)	
Ciplukan	29 (49,2)	
ACR EULAR 2013 Clinical Symptoms		
Finger fibrosis	59 (100)	
Raynaud's phenomenon	59 (100)	
Finger edema	54 (91,5)	
Finger scars	36 (61,0)	
Salt and Pepper Appearance	32 (54,0)	
Teleangiectasia	17 (28,8)	
MRSS		16 (6-36)
Laboratorium		
Hb (g/dL)		12,7 (10,5 – 15,1)
Leukocyte (/mm ³)		7.950 (4.490 – 19.400)
Trombocyte (/mm ³)		335.017 ± 90.136
LED (mm/jam)		38 ± 22
SGOT (U/L)		17 (11 -59)
SGPT (U/L)		13 (5 – 42)
Creatinine (mg/dL)		0,65 (0,28 -1,92)
Proteinuria	n=41	
Neg	36 (87,8)	
1+	5 (12,2)	
P1NP		50,6 (14,9-177,8)

Bivariate analysis

Data changes in P1NP and MRSS Baseline and

after treatment at the 4th, 8th and 12th weeks in this study are shown in Table 2 below.

Table 2. Changes in P1NP and MRSS baseline and after treatment at the 4th, 8th and 12th weeks

Variable	Baseline	4-weeks	8-weeks	12-weeks	P Value
Concentration					
P1NP (ng/mL)					
Median	50,6	45,0	42,0	39,2	0,033*
Min – Max	14,9 – 177,8	9,8 – 130,3	9,3 – 182	9 – 187,7	
Mean Rank	2,79	2,64	2,36	2,22	
MRSS					
Median	16	14	14	13	<0,001*
Min – Max	6 – 36	5 – 32	5 – 37	3 – 38	
Mean Rank	3,40	2,73	2,05	1,82	

Description: Analysis used Friedman test

From the results of the analysis, there was a downward trend in P1NP from baseline (week 0) and after treatment at the 4th, 8th and 12th weeks with a Median P1NP of 50.6 ng/mL, 45.0 ng/mL, 42.0 ng/mL and 39.2 ng/mL. Likewise in the MRSS there was a downward trend of change with the median

MRSS 16, 14, 14, 13, respectively.

The Friedman test analysis showed the mean rank decreased in both P1NP and MRSS with p-values of 0.033 and <0.001 (p<0.05).

The bar graph in Figure 1 shown the trend of change in mean rank values from baseline and after treatment at the 4th, 8th and 12th weeks.

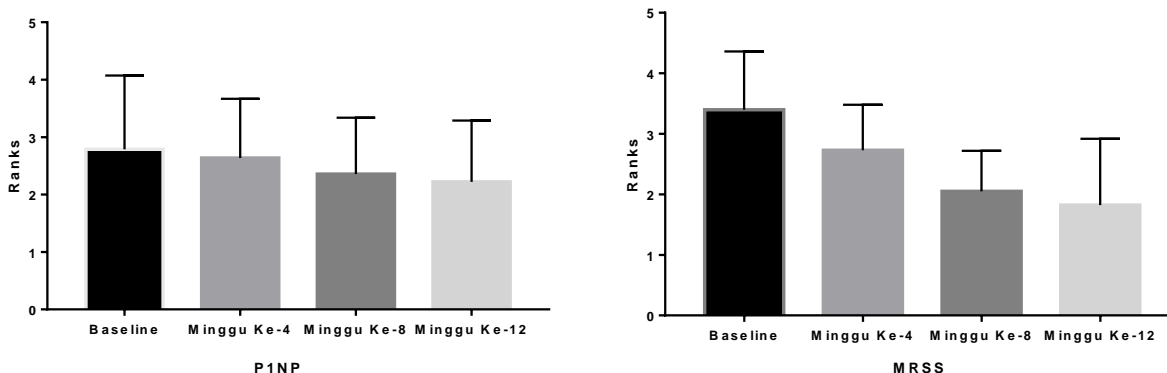


Figure 1 Bar graph of changes in baseline P1NP and MRSS and after treatment at the 4th, 8th and 12th weeks

The changes in baseline P1NP and MRSS and after treatment at the 4th, 8th and 12th weeks are shown in Table 3. Results of analysis in baseline P1NP and MRSS and after treatment at the 4th, 8th

and 12th weeks. indicates there was a significant difference in both P1NP and MRSS between the baseline examination and the 4th, 8th and 12th weeks after treatment.

Table 3. Differences in changes in baseline P1NP and MRSS and after treatment at the 4th, 8th and 12th weeks

	Variable	Median Difference (IK 95%)	Z	P Value	Eta squared (η^2)
Baseline vs. 4th weeks	Concentration P1NP (ng/mL)	2,8 (-2,2 – 8,3)	-2,006	0,022*	0,068
	MRSS	1 (1 – 2)	-4,289	<0,001*	0,312
Baseline vs. 8th weeks	Concentration P1NP (ng/mL)	4,9 (0 – 8,3)	-2,447	0,007*	0,101
	MRSS	3 (2 – 4)	-5,002	<0,001*	0,424
Baseline vs. 12th weeks	Concentration P1NP (ng/mL)	5,1 (-1,9 – 13,6)	-2,661	0,004*	0,120
	MRSS	4 (2 – 5)	-4,891	<0,001*	0,405
4th weeks vs. 8th weeks	Concentration P1NP (ng/mL)	0,5 (-1,5 – 3,3)	-0,840	0,201	0,012
	MRSS	1 (1 – 2)	-3,912	<0,001*	0,259
4th weeks vs. 12th weeks	Concentration P1NP (ng/mL)	1,8 (-0,6 – 4,9)	-1,808	0,036*	0,055
	MRSS	2 (1 – 3)	-3,624	<0,001*	0,223
8th weeks vs. 12th weeks	Concentration P1NP (ng/mL)	0,4 (-2,4 – 3,9)	-1,344	0,092	0,031
	MRSS	1 (0 – 2)	-2,033	0,021*	0,070

Description: CI=Confidence interval, analysis used Wilcoxon test

From the analysis results, overall showed that MRSS had a greater change effect than P1NP. From baseline to 4th weeks, the eta square value in P1NP was 0.068 while in MRSS was 0.312, means that P1NP only had a decreasing effect of 6.8% while in MRSS was greater, which was 31.2%.

The decrease in P1NP and MRSS based on

cutaneous classification is shown in Table 4. Friedman test analysis showed in limited type systemic sclerosis the mean rank only decreased in MRSS with a p value of <0.001, but in the diffuse type the mean rank decreased in both P1NP and MRSS with a p value of 0.033 and <0.001 (p<0.05).

Table 4. Changes in baseline P1NP and MRSS and after treatment 4th week, 8th week and 12th week based on cutaneous classification

Variable	Baseline	4 th weeks	8 th weeks	12 th weeks	p Value
521					
LIMITED TYPE SYSTEMIC SCLEROSIS (n=24)					
Concentration P1NP (ng/mL)					
Median	46,5	39,8	39,2	32,1	0,399
Min – Max	14,9 – 105,6	16,9 – 122,4	15,4 – 106,3	17,3 – 107,8	
Mean Rank	2,69	2,56	2,38	2,38	
MRSS					
Median	12	11	9	8	<0,001*
Min – Max	6 – 36	5 – 31	5 – 37	3 – 38	
Mean Rank	3,31	2,71	2,15	1,83	
DIFFUSED TYPE SYSTEMIC SCLEROSIS (n=35)					
Concentration P1NP (ng/mL)					
Median	54,0	49,2	45,7	42,2	0,033*
Min – Max	20,1 – 177,8	9,8 – 130,3	9,3 – 182	9,0 – 187,7	
Mean Rank	2,86	2,69	2,34	2,11	
MRSS					
Median	23	18	16	16	<0,001*
Min – Max	9 – 34	7 – 32	6 – 30	5 – 28	
Mean Rank	3,46	2,74	1,99	1,81	

Description: Analysis used Friedman test

4. Discussion

The results showed the mean age of the study subjects was 42 years with a range from 21 to 57 years, this corresponds to the highest onset of systemic sclerosis in the third and fourth decades.¹⁸ The mean age differs from the study conducted by

Denton et al.¹⁹ which is 48.5+11 years. The age difference in the two study populations may be caused racial differences where there are differences in autoantibody expression and genetic factors that play a role in the pathophysiology of systemic sclerosis in the Caucasian population compared to

the Asian population.¹⁵ The sex of the subjects were mostly women, which was 96.6%. This is consistent with the incidence of systemic sclerosis in general which occurs in women.^{18,20} This study conducted by Low et al.¹⁴ in Asia also get similar results where 86% of patients with systemic sclerosis are women.

The median baseline MRSS value in this study was 16 with a range of 6 to 36. MRSS as the gold standard for assessing the degree of fibrosis can be used to assess the course of the disease and the response of skin fibrosis to therapy. Monitoring of MRSS based on disease course from longitudinal studies shows that MRSS will change 3-6 months after therapy.⁸⁻¹⁰ The proximal areas of the body may return to normal structure over the course of treatment, but this does not occur in areas of the body with more severe fibrosis or atrophy, generally distal to the fingers.²⁶

The median baseline P1NP level in this study was 50.6 (14.9–177.8) ng/mL. Minier et al.²² showed almost the same level of 45.0 (34–65) ng/mL. This study did not examine P1NP controls in the normal population, but normal levels were obtained from the study of Minier et al.²²

The type of treatment of subjects in this study was not the same for every patient. Medication were given according to patient needs and drug availability. DMARD methotrexate was administered to 56 (94.9%) subjects. Mycophenolate mofetil (MMF) was administered to 3 (0.33%) subjects. Steroids were administered to 31 (52.5%) subjects. The number of subjects who received aspirin and clopidogrel therapy were 46 (77.9%) subjects and 7 (11.8%) subjects, respectively. 3 (5.1%) subjects were undergoing cyclophosphamide chemotherapy due to pulmonary involvement. Calcium channel blockers were given to 59 (100%) subjects due to complaints of Raynaud's phenomenon. In this study, there were data on 29 (49.2%) subjects who received ciplukan herbal therapy, which was given while participating in Dewi's study.¹⁷ The medications given were not homogeneous, but during the 3 months of

observation there was no change in the type of drugs in each patient, the patients received the same type of medication every month.

The results of the analysis showed that there was a downward trend in both MRSS and P1NP from baseline and after treatment at the 4th, 8th, and 12th weeks with Friedman Test analysis shown the mean rank decreased in both MRSS and P1NP with a p value <0.05. Similar results with Denton et al.¹⁹ and Kuhn et al.⁹ which showed improvement in MRSS after 3 months therapy as well as P1NP concentrations, and there was a significant relationship between P1NP and MRSS. Similar results study with Le EN et al.¹⁶ proves that changes in MRSS can be detected in the 3rd month of treatment, and similar with the study Ponticos et al.⁶⁹ proves that P1NP can be used as a monitoring response to therapy.

Disease activity in systemic sclerosis always changes with high activity at the beginning of the disease and fluctuates during the course of the disease and increases influenced by factors such as infection, stress, exposure to toxic substances and others. Tissue damage fibrosis in patients with systemic sclerosis can be permanent and improve with therapy, although improvement does not occur in a short time. Patients with diffuse systemic sclerosis have a rapid increase in MRSS early in their disease. Skin scores peak 12-18 months after the first symptoms of systemic sclerosis and decline slowly thereafter, but usually do not return to 0 (normal). Limited systemic sclerosis has a restricted distribution of crusting and thickening of the skin (on the fingers, back of the hand, and rarely the distal forearm) that does not spread, regardless of how long the disease has lasted. This type has fewer complications to internal organs with better survival. Internal organ involvement occurs slowly, often over decades.²⁴

Factors such as infection, stress, exposure to toxic substances that influence fluctuations in the course of the disease were not assessed during this

study. This study also did not distinguish between new patients who had not received therapy, or old patients who had received therapy, either on DMARD therapy, steroids, or standardized herbal medicines, and did not differentiate based on the stage of the disease and the clinical condition of the patient since the beginning of the study. This may have resulted in wide range of P1NP values in this study, with the lowest P1NP was 9 ng/mL and the highest was 187.7 ng/mL, contrast with study conducted by Minier et al. with P1NP range of 34-65 ng/mL.²²

Skin fibrosis in systemic sclerosis is an excessive deposition and accumulation of extracellular matrix, especially type 1 collagen, with P1NP as a metabolite of type 1 formation. P1NP concentrations may occur with clinical cutaneous fibrosis in systemic sclerosis. MRSS is a validated fibrosis assessment, especially when performed by an experienced rheumatologist. In this study, the MRSS assessment was done by experienced rheumatologist, this causes changes in detecting from the MRSS examination on skin thickness, made MRSS have a greater decrease in change than P1NP concentration.

The analysis results based on the cutaneous classification of systemic sclerosis (limited type and diffuse type) showed that in both the limited type and the diffuse type there was a trend of changes in P1NP and MRSS. ($p < 0.001$), but not significant in P1NP ($p = 0.399$). Meanwhile, in the diffuse type, both MRSS and P1NP experienced significant decrease ($p < 0.05$).

The overall effect of P1NP and MRSS-lowering changes was greater in the diffuse type of systemic sclerosis. This is following a research by Vincent et al.¹⁶ previously demonstrated that the diffuse type subjects showed a higher correlation between MRSS and P1NP which was not found in the limited type. This might be due to the fibrotic process that occurs in widespread diffuse type systemic sclerosis involved internal organs, resulting in higher concentrations of P1NP and MRSS compared to the restricted type. The complex pathophysiology and the difference in the course of the disease between the diffuse type and the

limited type may be the reason for the different changes in P1NP and MRSS concentrations.^{28,29}

This study did not distinguish between new patients who had never received therapy or the patients who had previously received therapy. In this study, the standard therapy given was also not entirely the same, both in type and dose, adjusted for the patient's clinical condition and drug availability. Different therapies can give different results. Dewi S et al.¹⁷ showed clinical improvement occurred more quickly in patients given ciplukan adjuvant therapy. This may also cause the difference in MRSS with P1NP changes during the study, so the MRSS has a greater decrease in change than P1NP.

This study showed both P1NP and MRSS had a statistically significant reduction at 3 months of treatment. Although the effect of changes in P1NP was smaller than MRSS, this difference in changes was not statistically significant ($p = 0.06$). P1NP can be considered to see the response of treatment in systemic sclerosis if there is no doctor trained for MRSS examination.

This study has several limitations that may affect the results of the study. Limitations in this study include: 1) The research subjects were mostly not naive patients, many confounding variables. Subjects were not differentiated based on already on treatment and naïve patients and their disease stage, thus disease activity could be different which would affect the MRSS and P1NP values. 2) Factors such as infection, stress, exposure to toxic substances that influence fluctuations in the course of the disease were not assessed during this study, changes in P1NP and MRSS did not fully reflect the response to therapy. 3) This study did not distinguish the type of therapy.

5. Conclusion

There was a difference in the decrease in P1NP concentrations and MRSS in systemic sclerosis at 3 months of treatment, MRSS had a greater decrease in change than P1NP at 3 months of treatment, but the

difference was not statistically significant.

Research on response to therapy based on MRSS with serum P1NP levels should be carried out in new patients who have not received therapy, either DMARD, steroids, or standardized herbal drugs, using a prospective cohort method by controlling for confounding variables. MRSS and P1NP can be used to assist clinicians in assessing the response of therapy. Training for MRSS examination is needed for internal medicine specialists to reduce the subjectivity of the examination results. P1NP can be considered to look for the response of treatment in systemic sclerosis if there are no doctors trained for MRSS examination in these patients.

6. References

1. Mayes M, Assasi S. Classification and Epidemiology of scleroderma. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. *Rheumatology (Oxford)*. 6th Ed. Philadelphia: Elsevier. 2015; 1153-7.
2. Hunzelmann N, Brinckmann J. What are the new milestones in the pathogenesis of systemic sclerosis? *Ann Rheum Dis*. 2010; 69 Suppl 1: i52-6.
3. Derk CT, Jimenez SA. Systemic sclerosis: current views of its pathogenesis. *Autoimmunity reviews*. 2003; 2(4):181-91.
4. Czirjak L, Foeldvari I, Muller-Ladner U. Skin involvement in systemic sclerosis. *Rheumatology (Oxford)*. 2008; 47(5): 44-5.
5. Furst DE, Clements PJ, Steen VD, Medsger TA, Jr., Masi AT, D'Angelo WA, et al. The modified Rodnan skin score is an accurate reflection of skin biopsy thickness in systemic sclerosis. *J Rheumatol*. 1998; 25(1): 84-8.
6. Clements PJ, Hurwitz EL, Wong WK, Seibold JR, Mayes M, White B, et al. Skin thickness score as a predictor and correlate of outcome in systemic sclerosis: high-dose versus low-dose penicillamine trial. *Arthritis Rheum*. 2000; 43(11): 2445-54.
7. 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. *Ann Rheum Dis*. 2007; 66(7): 966-9.
8. Le EN, Wigley FM, Shah AA, Boin F, Hummers LK. Long-term experience of mycophenolate mofetil for treatment of diffuse cutaneous systemic sclerosis. *Ann Rheum Dis*. 2011; 70(6): 1104-7.
9. Kuhn A, Haust M, Ruland V, Weber R, Verde P, Felder G, et al. Effect of bosentan on skin fibrosis in patients with systemic sclerosis: a prospective, open-label, non-comparative trial. *Rheumatology (Oxford)*. 2010; 49(7): 1336-45.
10. Amjadi S, Maranian P, Furst DE, Clements PJ, Wong WK, Postlethwaite AE, et al. Course of Modified Rodnan Skin Score in Systemic Sclerosis Clinical Trials: Analysis of 3 Large Multicenter, Randomized Clinical Trials. *Arthritis Rheum*. 2009; 60(8): 2490-8.
11. Affandi AJ, Radstake TR, Marut W. Update on biomarkers in systemic sclerosis: tools for diagnosis and treatment. *Semin Immunopathol*. 2015; 37(5): 475-87.
12. Minier T. Assessment of Disease Activity and Evaluation of Clinical Parameters and Biomarkers in Systemic Sclerosis: University of Pecs; 2011.
13. Reveille JD. Ethnicity and race and systemic sclerosis: how it affects susceptibility, severity, antibody genetics, and clinical manifestations. *Curr Rheumatol Rep*. 2003; 5(2): 160-7.
14. Ling ALH, Gee TG, Giap LW, Cheng NS, Santosa A, Chan G, et al. Disease Characteristics of the Singapore Systemic Sclerosis Cohort. *Proceedings of Singapore*

- Healthcare. 2013; 22(1): 8-14.
15. Mulla E, Shaffu S, Hassan W. A Comparative Study Of The Difference In Clinical Manifestations And Disease Outcome Between South Asian And Caucasian Patients With Systemic Sclerosis In A Large NHS Trust, Within The United Kingdom. *Ann Rheum Dis.* 2015; 75: 1128.
 16. Vincent, Dewi S, Wachyudi RG. Correlation between serum procollagen type 1 N-Terminal Propeptide Level With Modified Rodnan Skin Score In Systemic Sclerosis Patients. *Ina J Rheum.* 2017; 9(2): 8-12
 17. Dewi S, Isbagio H, Purwaningsih EH, Kertia N, Setiabudy R, Setiati S. A Double-blind, Randomized Controlled Trial of Ciplukan (*Physalis angulata* Linn) Extract on Skin Fibrosis, Inflammatory, Immunology, and Fibrosis Biomarkers in Scleroderma Patients. *Acta Med Indones* 2019;51(4):303-310.
 18. Varga J, Lafyatis R. Etiology and pathogenesis of systemic sclerosis. *Rheumatology (Oxford).* 6th Ed. Philadelphia: Elsevier; 2015; 1177-85.
 19. Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, et al. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. *Arthritis Rheum.* 2007; 56(1): 323-33.
 20. Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *N Engl J Med.* 2009; 360(19): 1989-2003.
 21. Denton CP, Black CM, Korn JH, de Crombrughe B. Systemic sclerosis: current pathogenetic concepts and future prospects for targeted therapy. *The Lancet.* 347(9013): 1453-8.
 22. Minier T, Nagy Z, Balint Z, Farkas H, Radics J, Kumanovics G, et al. Construct validity evaluation of the European Scleroderma Study Group activity index, and investigation of possible new disease activity markers in systemic sclerosis. *Rheumatology (Oxford).* 2010; 49(6): 1133-45.
 23. Kowal-Bielecka O, Fransen J, Avouac J, Becker M, Kulak A, Allanore Y, et al. Update of EULAR recommendations for the treatment of systemic sclerosis. *Ann Rheum Dis.* 2017; 76(8): 1327-1339.
 24. Varga J. From Pathogenesis to Comprehensive Management. 2nd Ed. new york: Springer. 2017.
 25. Khanna D. Measuring Disease Activity and Outcome in Clinical trials. In: Varga J, Denton CP, Wigley FM, editor. *Scleroderma From Pathogenesis to Comprehensive Management.* New York: Springer. 2012; 661-4.
 26. Varga J. Systemic sclerosis: epidemiology, pathology and pathogenesis. *Primer on rheumatic diseases* 13th ed Atlanta: Springer. 2008: 351-8.
 27. Hunzelmann N, Risteli J, Risteli L, Sacher C, Vancheeswaran R, Black C, et al. Circulating type I collagen degradation products: a new serum marker for clinical severity in patients with scleroderma? *Br J Dermatol.* 1998; 139(6): 1020-5.
 28. Scheja A, Wildt M, Wollheim FA, Akesson A, Saxne T. Circulating collagen metabolites in systemic sclerosis. Differences between limited and diffuse form and relationship with pulmonary involvement. *Rheumatology (Oxford).* 2000; 39(10): 1110-3.
 29. Varga J. Overview: Pathogenesis Integrated. In: Varga J, denton C, Wigley FM, editor. *Scleroderma From Pathogenesis to Comprehensive Management.* New york: Springer. 2012; 163-4.