Serum levels of soluble ST2 and interleukin-33 in patients with dermatomyositis and polymyositis

L. Yuan[1], L. Yao[2], L. Zhao[1], L. Xia[1], H. Shen[1], J. Lu[1]

[1]Department of Rheumatology, First Affiliated Hospital of China Medical University; [2]China Medical University, Shenyang, China.

Abstract

Objectives

The purpose of this study is to determine whether soluble ST2 (sST2) and interleukin (IL)-33 is involved in dermatomyositis (DM) and polymyositis (PM).

Methods

Serum sST2 and IL-33 levels in 49 DM and 21 PM were detected by enzyme-linked immunosorbent assay (ELISA). Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), creatine kinase (CK), lactate dehydrogenase (LDH) and antinuclear antibody (ANA), anti-Jo-1 antibody and anti-Mi-2 antibody were tested by standard laboratory techniques. Interstitial lung disease (ILD) was identified on high-resolution computed tomography (HRCT). The visual analogue scale (VAS) of the disease activity, muscle strength, lung functional parameters and other clinical features of DM/PM patients were recorded as well.

Results

Sera sST2 levels were significantly higher in DM and PM patients and correlated with CRP, CK, LDH and VAS. The level of serum sST2 decreased after therapy. Conversely, serum levels of IL-33 in patients with PM and DM were not significantly higher than those from HC.

Conclusion

The level of sST2 is elevated in sera of DM and PM patients. sST2 levels were correlated with other markers of disease activity. This data support that sST2 may play a role in DM and PM.

Key words

dermatomyositis, polymyositis, soluble ST2, interleukin-33

Lin Yuan, MSc
Lutian Yao, MSc
Lin Zhao, MSc
Liping Xia, MD
Hui Shen, MD
Jing Lu, MD

Please address correspondence to:
Dr Lu Jing,
Department of Rheumatology and Immunology,
First Affiliated Hospital of China Medical University,
155 Nanjing North Street,
Heping District,
110001 Shenyang, PR China.
E-mail: lujingtan@163.com

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Introduction

Dermatomyositis (DM) and polymyositis (PM) both belong to the idiopathic inflammatory myopathies (IIMs), which are chronic autoimmune disorders sharing the clinical symptom of muscle weakness (cutaneous legions in DM) and inflammatory cell infiltrates in muscle tissue. Proinflammatory cytokines and chemokines have been found in muscle tissue of DM and PM patients.

Interleukin (IL)-33 is a new member of the IL-1 family. It plays a key role in mediating Th2 immune responses and promoting the pathogenesis of Th2-related disease (1-4). ST2 is a member of the IL-1 receptor family and was discovered to be specific ligand of IL-33 (1-4). There were three different splice variants of ST2: ST2L, soluble ST2 (sST2) and ST2V. IL-33 stimulates target cells by binding to ST2, thereby activating nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) pathways (1). sST2 acts as a decoy receptor that prevents the interaction of ST2L with IL-33 (1).

Previous studies showed that IL-33/sST2 may play an important role in the development of autoimmune diseases such as rheumatoid arthritis (RA), allergic rhinitis, and systemic lupus erythematosus (SLE) (5-7). Mok *et al.* found that sST2 is increased in SLE patients and is correlated with disease activity (7). However, the roles of IL-33/sST2 in DM and PM are unknown. In this study, we investigate the levels of IL-33/sST2 and association with clinical parameters in the patients with DM and PM.

Materials and methods

Patients

Forty-nine patients with DM (38 women and 11 men; mean age 50.4±15.4 years) and 21 patients with PM (17 women and four men; mean age 55.5±15.4 years) were recruited. Diagnosis was based on Bohan and Peter criteria (8, 9). Thirty HC were also recruited (23 women and seven men; mean age 53.2±15.7 years), whose age and gender were matched to the patient cohort. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983. All patients gave written, informed consent for participation in the study.

Disease activity evaluations and other clinical characteristics

Disease activity was evaluated by the visual analogue scales (VAS) score developed by the International Myositis Assessment and Clinical Studies (IMACS) Group (10). Clinical parameters were assessed, including disease duration, muscle strength, lung functional parameters and medications. Laboratory tests, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), creatine kinase (CK), lactate dehydrogenase (LDH) and antinuclear antibody (ANA), anti-Jo-1 antibody and anti-Mi-2 antibody were measured by standard methods. In addition, interstitial lung disease (ILD) was identified on high-resolution computed tomography (HRCT).

Sera IL-33 and sST2 levels assays

Sera levels of IL-33 and sST2 were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's directions (R&D systems, Minneapolis, MN, USA). The detection limits were IL-33, 31.25pg/ml; sST2, 23.44pg/ml.

Statistical analysis

Data were processed to calculate median or the mean±SD. Continuous variables from the study were analysed by the ANOVA and/or the Student's *t*-test with a Gaussian population or the Mann-Whitney U-test with a non-Gaussian population. Spearman's correlation coefficient was used to test the correlations between two variables. All analyses were performed by using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and GraphPad 5 software. Differences of $p<0.05$ were considered significant.

Results

Clinical characteristics of DM/PM patients

Demographic and clinical characteristics of DM and PM patients are shown

in Table I. Among the 70 patients, 37 (52.9%) were ANA-positive, 14 (20.0%) were anti-Mi-2 antibody positive, 10 (10.7%) were anti-Jo-1 antibody positive and 35 (50.0%) had ILD.

Serum levels of sST2 and IL-33 in DM and PM patients and HC

Serum levels of sST2 in PM (median, [25–75 percentiles], pg/ml: 414.1, [191.9–1272.0]) and DM (median, [25–75 percentiles], pg/ml: 671.2, [298.1–2205.0]) patients were higher than those in HC (median, [25–75 percentiles], pg/ml: 193.7, [108.1–250.3]) ($p<0.0001$ and $p<0.0001$, respectively) (Fig. 1.A1). In contrast, serum levels of IL-33 in patients with DM and PM were not significantly elevated compared with HC ($p>0.05$), (Fig. 1.A2). There were no significant differences in serum sST2 and IL-33 levels between DM and PM patients ($p>0.05$).

Correlation of IL-33 and sST2 with clinical parameters and disease activity

Serum IL-33 levels were not correlated with ESR, CRP, CK, LDH, muscle strength and VAS in DM and PM patients. In contrast, serum sST2 levels were correlated significantly with CRP (r=0.6133, $p<0.0001$) (Fig. 1.B1), CK (r=0.3123, $p<0.05$), LDH (r=0.6334, $p<0.0001$) (Fig. 1.B2) and VAS (r=0.5888, $p<0.0001$) in DM patients (Fig. 1.B3). And in patients with PM, the sST2 values were also found to be associated with CRP (r=0.4563, $p<0.05$) (Fig. 1.C1), CK (r=0.4512, $p<0.05$), LDH (r=0.5748, $p<0.01$) (Fig. 1.C2) and VAS (r=0.4804, $p<0.05$) (Fig. 1.C3). But there was no correlation between levels of sST2 and muscle strength both in DM and PM patients.

Decreased serum levels of sST2 after therapy

Among the 70 patients, 15 (12DM and 3PM) were treated naïve. After the blood samples collected, all 15 patients were treated immediately by 0.5–1.0mg/kg prednisone and DMARDs that included methotrexate, cyclophosphamide, azathioprine. Sera samples were collected again after a 6-month therapy. The serum levels of sST2 were significantly decreased after therapy ($p<0.05$), (Fig. 1.D).

Table I. Clinical characteristics in patients with PM/DM.

Demographics and laboratory characteristics	PM (n=21)	DM (n=49)	HC (n=30)
Sex, female n (%)	17 (80.1)	38 (77.6)	23 (76.6)
Age, mean (SD), years	55.5 (15.4)	50.4 (15.4)	53.2 (15.7)
Disease duration, median, months	18.0 (6.0–48.0)	6.0 (3.0–24.0)	–
Initial treatment n (%)	14 (66.7)	28 (57.1)	–
Serological features			
ESR, median (25–75 percentiles), mm	24.0 (10.0–52.0)	25.0 (10.5–47.5)	–
CRP, median (25–75 percentiles), mg/L	15.3 (4.765–20.1)	7.7 (3.3–17.6)	–
CK, median (25–75 percentiles), U/L	617.0 (157.0–2204.0)	93.0 (47.5–1555.0)	–
LDH, median (25–75 percentiles), U/L	469.0 (280.0–599.0)	353.0 (231.5–582.0)	–
VAS, median (25–75 percentiles)	6.0 (4.0–7.0)	6.0 (4.0–8.0)	–
ANA-positive, n (%)	11 (52.3)	26 (53.1)	–
Mi-2 antibody-positive, n (%)	3 (14.3)	11 (22.4)	–
Jo-1 antibody-positive, n (%)	7 (33.3)	3 (6.1)	–
With ILD, n (%)	12 (57.1)	23 (46.9)	–
FEV1/FVC, mean (S.D.) (%)	64.5 (8.3)	61.5 (5.9)	–
FEV1/FEV with ILD, mean (SD) (%)	64.3 (7.9)	62.1 (6.5)	–
DLCO, mean (SD)	19.0 (3.8)	20.6 (2.9)	–
DLCO with ILD, mean (SD)%	18.8 (3.5)	19.3 (3.1)	–
PM/DM with skin lesions, n (%)	-	38 (77.5)	–
Muscle strength, median (25–75 percentiles)	4.0 (4.0–5.0)	5.0 (4.0–5.0)	–
Treatment			
Steroid, n (%)	21 (100)	49 (100)	–
CTX, n (%)	2 (10)	8 (16)	–
AZA, n (%)	5 (24)	14 (28)	–
MTX, n (%)	5 (24)	6 (12)	–
HCQ, n (%)	0 (0)	12 (24)	–

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; CK: creatine kinase; LDH: lactate dehydrogenase; VAS: visual analogue scale; ANA: antinuclear antibody; ILD: interstitial lung disease; DLCO: carbon monoxide diffusing capacity; FEV1: forced expiratory volume in one second; CTX: cyclophosphamide; AZA: azathioprine; MTX: methotrexate; HCQ: hydroxychloroquine.

Levels of serum IL-33/sST2 in DM/PM patients with specific auto-antibodies and organ involvement

IL-33 and sST2 levels were not significantly different between auto antibodies positive or negative groups, including ANA, anti-Mi-2 and anti-Jo-1. There was no significant difference in IL-33 and sST2 levels in DM/PM patients with or without ILD either. Moreover, IL-33 and sST2 levels did not correlate with FEV1% and DLCO. (Table II).

Discussion

DM and PM are chronic autoimmune disorders sharing the clinical symptom of muscle weakness and inflammatory cell infiltrates in muscle tissue. Data showed that inflammatory cells, including $CD4^+$, $CD8^+$ T lymphocytes, were activated in DM/PM patients, not only in the skeletal muscle, but also in peripheral blood (11, 12). Th1 and Th17 proinflammatory cytokines that present in myositis tissues are associated with the migration, differentiation, and maturation of inflammatory cells (13, 14). Previous studies showed that sST2 was correlated positively with disease activity in systemic lupus erythaematosus (SLE) (7), ulcerative colitis (15) and RA (Xiaolin L, Jing L, unpublished data). And sST2 was also found to correlate positively with severity in CK in patients with myocardial infarction and it has also been reported to be of prognostic value in the prediction of cardiac mortality in patients with acute myocardial infarction (16). In this report, we showed the first detailed analysis of sST2 and IL-33 levels in sera of DM and PM patients. Interestingly, we observed significantly higher levels of sST2 in DM/PM patients compared to healthy controls. To our knowledge, the involvement of sST2 in DM and PM was previously unreported. In this study, we also showed the significant correlation between serum sST2 levels and other clinical parameters, including CRP, CK and LDH. Furthermore,

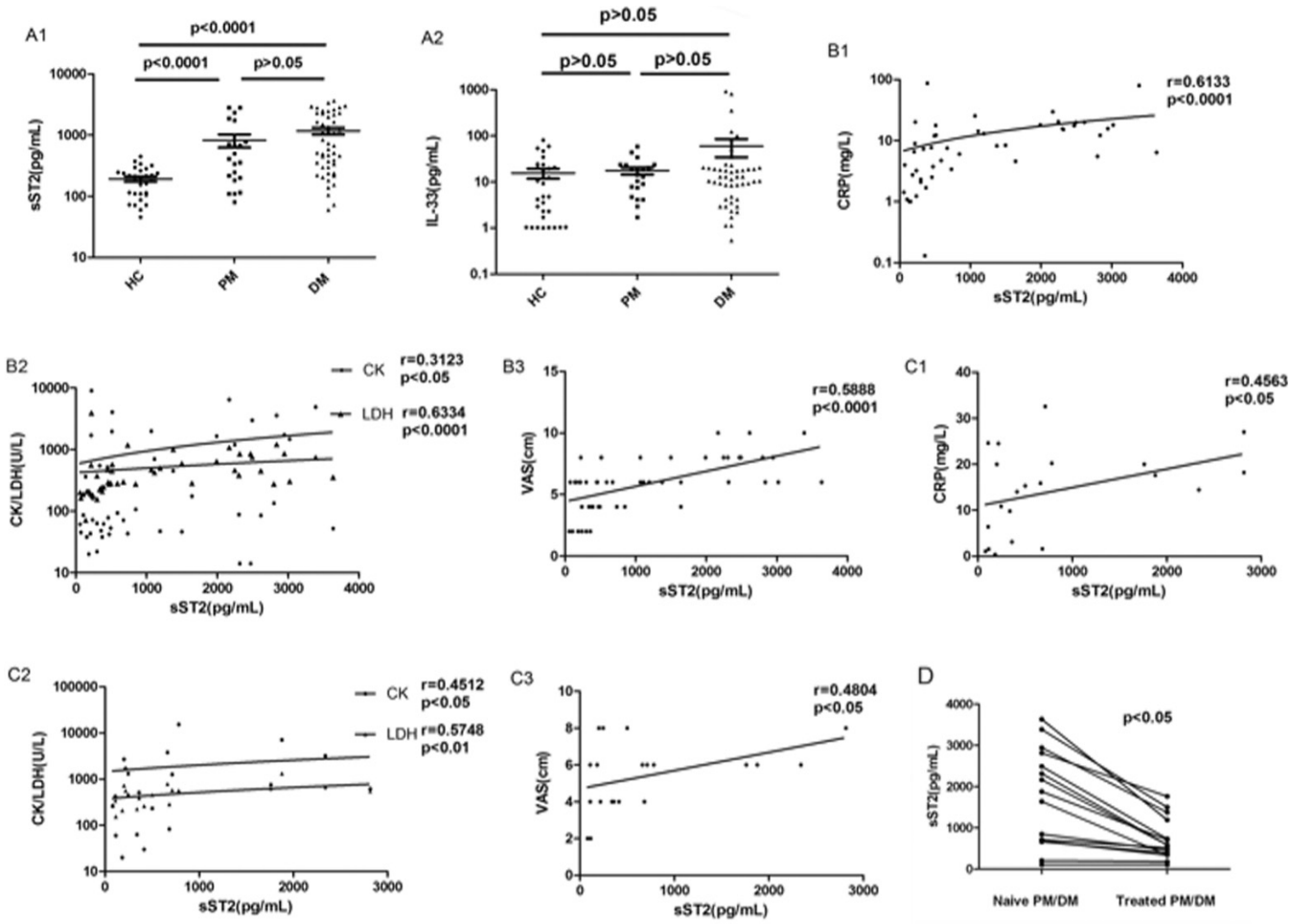

Fig. 1. **A1**. The serum levels of sST2 in PM patients, DM patients and healthy controls. **A2**. The serum levels of IL-33 in PM patients, DM patients and healthy controls. **B1**. The correlation between the serum levels of sST2 and CRP in DM patients. **B2**. The correlation between the serum levels of sST2 and CK or LDH in DM patients. **B3**. The correlation between the serum levels of sST2 and VAS in DM patients. **C1**. The correlation between the serum levels of sST2 and CRP in PM patients. **C2**. The correlation between the serum levels of sST2 and CK or LDH in PM patients. **C3**. The correlation between the serum levels of sST2 and VAS in PM patients. **D**. The serum levels of sST2 before and after therapy in PM and DM patients.

Table II. Specific auto-antibodies and organ involvement in patients with PM/DM.

		IL-33, median (25–75 percentile)		sST2, median (25–75 percentile)	
		(pg/ml)	*p*-value	(pg/ml)	*p*-value
PM (n=21)	ANA-positive	9.0 (4.1–19.7)		500.4 (338.7–1762.0)	
	ANA- negative	20.0 (9.1–23.7)	*p*>0.05	191.9 (109.1–1291.0)	*p*>0.05
	Mi-2 antibody-positive	6.6 (3.2–17.1)		1293.0 (224.7–2687.0)	
	Mi-2 antibody- negative	19.7 (7.7–23.7)	*p*>0.05	414.1 (148.7–747.5)	*p*>0.05
	Jo-1 antibody-positive	16.4 (7.1–30.0)		581.6 (400.7–2816.0)	
	Jo-1 antibody- negative	14.4 (4.1–23.7)	*p*>0.05	246.2 (114.4–782.8)	*p*>0.05
	with ILD	19.7 (6.1–29.0)		662.7 (269.8–2108.0)	
	without ILD	9.7 (5.1–22.8)	*p*>0.05	231.9 (110.4–636.8)	*p*>0.05
DM (n=49)	ANA-positive	17.6 (7.3–20.4)		677.9 (282.9–2258.0)	
	ANA- negative	9.2 (4.1–19.8)	*p*>0.05	671.2 (337.2–1638.0)	*p*>0.05
	Mi-2 antibody-positive	16.4 (8.6–23.0)		1497.0 (204.4–2315.0)	
	Mi-2 antibody- negative	12.2 (4.1–20.0)	*p*>0.05	629.1 (298.2–2051.0)	*p*>0.05
	Jo-1 antibody-positive	9.2 (1.7–122.3)		220.4 (182.9–2166.0)	
	Jo-1 antibody- negative	13.1 (4.6–20.0)	*p*>0.05	701.6 (327.4–2258.0)	*p*>0.05
	with ILD	14.4 (8.7–20.0)		590.7 (298.1–2224.0)	
	without ILD	13.1 (3.5–20.2)	*p*>0.05	731.9 (287.3–1971.0)	*p*>0.05

ILD: interstitial lung disease; ANA: antinuclear antibody.

we showed the strong positive correlations between sST2 levels and global VAS score both in DM and in PM patients. This indicated that sST2 may partly contribute to the activity of the DM and PM. However, the exact role of sST2 in the pathogenesis of DM and PM need to be clarified further.

IL-33/ ST2 acts as a pro-inflammatory cytokine which can mediate various immune responses (17). Our previous study showed elevated serum levels of IL-33 in RA patients, especially in RA patients with ILD (18). To our surprise, in this study, we did not show the difference in serum IL-33 levels between DM/PM patients and healthy controls. Furthermore, serum IL-33 levels were not related with other clinical parameters and VAS score in DM/PM patients. The reason for this may be that higher

level of sST2 can combine with IL-33 and decrease the serum levels of IL-33 in patients with DM and PM.

In the meantime, we did not find the relevance between IL-33/sST2 and auto-antibodies production. First of all, it may be because sST2 acts as a decoy receptor and higher levels of sST2 can prevent the interaction of ST2L with IL-33. Second of all, the positive rates of special auto-antibodies, including ANA, anti-Mi-2 and anti-Jo-1 antibodies were relatively low in DM and PM patients. Finally, the number of patients is not large enough to find the correlation between cytokines levels and antibody production.

In conclusion, high levels of sST2 provide additional evidence for activation of inflammatory response in patients with DM/PM. This suggested possible therapeutic significance of sST2 in patients with DM and PM.

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