Original article

Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus

Mo Yin Mok1, Fang Ping Huang2, Wai Ki Ip3, Yi Lo1, Fung Yi Wong3, Eric Yuk Tat Chan3, Kwok Fai Lam4 and Damo Xu5

Abstract

Objective. IL-33 has recently been found to be the specific ligand of ST2, an IL-1 receptor family member that is selectively expressed in Th2 cells and mediates Th2 response. This study aims to measure the serum levels of soluble ST2 (sST2) and IL-33 in patients with SLE and to examine their association with disease activity.

Methods. Seventy SLE patients were evaluated for disease activity, determined by SLEDAI, levels of anti-dsDNA antibody, C3 and C4. Fifty-seven patients were evaluated longitudinally on a second occasion. IL-33 and sST2 were measured by sandwich ELISA in the 127 SLE serum samples and compared with 28 age- and sex-matched healthy controls.

Results. Serum sST2 level was significantly higher in active SLE patients [0.51 (0.18) ng/ml] compared with inactive patients [0.42 (0.08) ng/ml] (P = 0.006) and normal controls [0.36 (0.13) ng/ml] (P < 0.001). sST2 level correlated significantly with SLEDAI, anti-dsDNA antibody and prednisolone dosage, and negatively with C3. Linear regression analysis showed that serum sST2 level was an independent predictive factor for modified SLEDAI, excluding anti-dsDNA and complement score after controlling for age, sex, glomerular filtration rate and prednisolone dosage (regression coefficient: 8.5; 95% CI 2.6, 14.3) (P = 0.005). Serum sST2 level was sensitive to change in disease activity longitudinally, with an effect size of 0.29. Elevated serum IL-33 was comparable in frequency (4.3 vs 7.1%; P = 0.62) and levels (P = 0.53) between SLE patients and controls.

Conclusions. Elevated serum sST2 level in SLE patients was found to correlate with disease activity and was sensitive to change, suggesting a potential role as a surrogate marker of disease activity.

Key words: Interleukin-33, Soluble ST2, Systemic lupus erythematosus disease activity index, T helper 2 immune response.

Introduction

IL-33 is a novel cytokine that belongs to the IL-1 family [1]. IL-33 has recently been found to be involved in the pathogenesis of chronic inflammatory arthritis [2] like its other family members, IL-1 and -18 [3]. It has also been described as a modulator of inflammation, mediating Th2 immune responses. IL-33 has been shown to induce production of IL-5, -13 and hypergammaglobulinaemia, typical of Th2-driven hyper-responsiveness [4]. It is also recognized to possess a chemoattractant effect for human Th2 cells [5]. IL-33 has been linked to allergic conditions as IL-33 has been shown to induce eosinophilic degranulation and IgE response [6] and production of ILs and chemokines by mast cells [7]. Administration of IL-33 into mice induced airway hyper-responsiveness and goblet cell hyperplasia [8].

ST2, receptor for IL-33 and a member of the IL-1 receptor family, was characterized over a decade before IL-33 was discovered to be its specific ligand [9]. ST2 is found
to be selectively expressed on a subset of Th2 cells but not Th1 cells and mediates important effector Th2 functions [9, 10]. IL-33 stimulates target cells by binding to ST2, thereby activating nuclear factor (NF)-κB and mitogen-activated protein kinase pathways [1]. ST2 is known to exist in a transmembrane form (ST2L) and is also alternatively spliced to produce a secreted soluble form (sST2) [11, 12]. Elevated serum sST2 has been reported in sepsis [13], asthma [14] and acute myocardial infarction [15], and the level of sST2 was found to correlate with the activity and severity of these medical conditions.

SLE is a multi-systemic autoimmune disease, characterized by hyper gammaglobulinaemia and a plethora of autoantibodies. Both Th1 and Th2 responses have been implicated in the pathogenesis of lupus [16–21]. While the Th2 type of response may be important in driving B-cell hyperactivity, cytokines that mediate Th1 responses have also recently been shown to be involved in the induction of pathogenic autoantibody isotypes leading to tissue pathology [21]. The enhanced expression of Th2 types of cytokines such as IL-10 may, therefore, reflect a desperate attempt of the immune system to down-regulate the pathogenic Th1 responses. Although the serum sST2 level has previously been found to be elevated in patients with SLE and other rheumatic diseases [22], its causal relationship with disease activity is still unclear. The serum level of its ligand, IL-33, has also not been described in SLE before and information with regard to the role of the IL-33/ST2 system and their clinical association in lupus is lacking. Our study aims to examine serum levels of IL-33 and sST2 in relation to disease activity and clinical association in patients with SLE.

Patients and methods

Patients and controls

The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Patients who satisfied the revised classification criteria of the ACR for SLE [23] were recruited from a university-affiliated Lupus clinic. Age- and sex-matched volunteers were recruited from the staff clinic as normal controls. Informed consent was obtained from recruited subjects. All subjects who had known allergic diseases were excluded. Patient information with regard to demographic data, cumulative clinical features, serological profile and medications were retrieved from medical records. Physical examination and laboratory investigations including complete blood count, liver and renal functions, levels of anti-dsDNA antibody, complements C3 and C4 were performed at study visits. Disease activity was determined according to the SLEDAI [24]. Active lupus disease was defined as SLEDAI > 4. Patients who developed significant proteinuria > 0.5 g/day with active urinary sediments or renal biopsy-proven lupus nephritis at the time of study were regarded as having active renal disease. Renal biopsy-proven lupus nephritis was interpreted according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification criteria [25]. Haematological involvement was defined as the presence of autoimmune haemolytic anaemia, leucopenia with white blood cell count < 3.0 x 10^9/l or thrombocytopenia with platelet count < 150 x 10^9/l. Renal function was estimated by estimated glomerular filtration rate (eGFR) using the modification of diet in renal disease (MDRD) formula, where eGFR = 186 x serum creatinine^−1.154 x age^−0.203 x (0.742 for female) [26]. Impaired renal function was defined as eGFR < 60 ml/min/1.73 m^2 [27]. Serum level of anti-dsDNA antibody was measured by in house ELISA using a calibration curve generated from six standards which were prepared with reference to the WHO international standard serum Wo80. The interassay coefficient of variation of the immunoassay for low and high controls was 14.7 and 6.7%, respectively. Serum C3 and C4 levels were determined by immunonephelometry (Immage 800; Beckman Coulter, California, USA). The lower normal limits were defined as 76 and 9 mg/dl, respectively. Some patients were evaluated longitudinally on two study visits for disease activity, serological features and serum sST2 level. Change in disease activity between the first and second occasions was defined as an increase or decrease in SLEDAI by > 4.

Assay of human IL-33, sST2

Ten millilitres of peripheral blood were collected from patients and controls, and stored at −70 °C for subsequent measurement of IL-33 and sST2. Serum level of IL-33 was measured by commercial sandwich ELISA (GenWay Biotech, San Diego, USA). Briefly, an ELISA plate was coated with capture antibody (affinity-purified chicken anti-human IL-33 mAb) in 0.05 M carbonate-bicarbonate followed by blocking. Serum samples were then added followed by horseradish peroxidase (HRP)-conjugated secondary mAb. Tetramethylbenzidine was subsequently added to the reaction which was stopped by applying 2 M H2SO4. Optical density was measured by microtitre plate reader at 450 nm. Serum level of IL-33 was read off from a standard curve according to the manufacturer’s instruction. Serum level of sST2 was measured by commercial sandwich ELISA (MBL). The capture antibody and the HRP-conjugated detection antibody involve two anti-human ST2 mAbs that recognize two different epitopes. The sensitivity of the immunoassays for IL-33 and sST2 are 0.7 and 0.032 ng/ml, respectively.

Statistical analysis

Data analysis was performed by SPSS 16.0 (Chicago, IL, USA). Data were summarized and presented as mean (s.d.) unless otherwise stated. The Mann–Whitney U-test and analysis of variance (ANOVA), where appropriate, were used to compare continuous variables between groups. Spearman’s correlation was used for correlation analysis. Paired non-parametric Wilcoxon signed-rank test was performed to compare serial changes in serum sST2 level. The effect size of sensitivity to changes was quantified by Cohen’s d. Fisher’s exact test was used to
compare the proportions of different groups. Logistic regression analysis was performed using active/inactive lupus disease status as dependent variable, with predictive factors identified in univariate analysis with $P < 0.1$ as independent variables. The contribution of each individual independent variable was expressed as odds ratio (OR) together with 95% CI for the association. As SLEDAI is a composite score involving components including anti-dsDNA antibody, C3 and C4, a multiple regression analysis was also performed with a modified SLEDAI score that excluded elevated anti-dsDNA antibody, low C3 and C4, as the dependent variable. Since the biochemical properties of sST2 in relation to age, sex and renal clearance are not known, the contribution of known biomarkers including anti-dsDNA antibodies, low C3 and C4 as well as serum sST2 level to modified SLEDAI score were analysed by adjusting for other variables including age, sex and eGFR. $P < 0.05$ was considered statistically significant in this study.

**Results**

**Clinical characteristics of SLE patients**

Seventy (67 females and 3 males) SLE patients of Southern Chinese origin were recruited. The mean (s.d.) age of these patients was 44.0 (11.0) years and they had been Followed up at the Lupus clinic for 13.9 (7.9) (median 13.0) years. Among these 70 SLE patients, 57 were evaluated longitudinally on a second occasion providing a total of 127 sets of clinical data and serum samples for subsequent analysis. Table 1 shows the demographics and clinical features of these patients at the time of the first and second clinical evaluations. Thirty-seven and 25 patients had active disease at the time of first and second assessment, respectively. Clinical features of disease exacerbation at the time of study were dominated by renal involvement (35/127, 27.6%) followed by non-renal exacerbations including cutaneous and articular manifestations (32/127, 25.2%) and haematological involvement (16/127, 12.6%). Among the 35 patients who had renal involvement, renal biopsy was performed in 13 patients. Two patients had ISN/RPS Class III, seven had Class IV alone and four patients had mixed Class IV and V lupus nephritis. Fifty-one and 42 patients were receiving oral prednisolone at a mean (s.d.) daily dose of 9.1 (7.6) and 6.8 (5.6) mg, respectively, for the first and second evaluation.

**Serum levels of IL-33 and sST2**

Serum levels of IL-33 and sST2 were measured in 70 SLE patients and 28 age- and sex-matched controls. Serum IL-33 level in most SLE patients was found to lie below the

---

**Table 1** Cumulative clinical features and clinical manifestations at the time of study of SLE patients

<table>
<thead>
<tr>
<th>Demographics and clinical characteristics</th>
<th>SLE ($n = 70$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female: male</td>
<td>67:3</td>
</tr>
<tr>
<td>Age at study, mean (s.d.), years</td>
<td>44.0 (11.0)</td>
</tr>
<tr>
<td>Duration of disease, mean (s.d.), years</td>
<td>13.9 (7.9)</td>
</tr>
<tr>
<td>Clinical manifestations, $n$ (%)</td>
<td></td>
</tr>
<tr>
<td>At the first evaluation ($n = 70$)</td>
<td></td>
</tr>
<tr>
<td>Arthritis/arthralgia</td>
<td>6 (8.6)</td>
</tr>
<tr>
<td>Oral ulcer</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Malar rash</td>
<td>7 (10.0)</td>
</tr>
<tr>
<td>Cutaneous vasculitis</td>
<td>3 (4.3)</td>
</tr>
<tr>
<td>Serositis</td>
<td>0</td>
</tr>
<tr>
<td>Autoimmune haemolytic anaemia</td>
<td>0</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>3 (4.3)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>51 (72.9)</td>
</tr>
<tr>
<td>Immune thrombocytopenia</td>
<td>3 (4.3)</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>25 (35.7)</td>
</tr>
<tr>
<td>Nervous system involvement</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>SLEDAI score, mean (median, range)</td>
<td>5.5 (6.0, 0–22)</td>
</tr>
<tr>
<td>Active disease (SLEDAI $&gt; 4$)</td>
<td>37 (52.9)</td>
</tr>
<tr>
<td>Serological features, $n$ (%)</td>
<td></td>
</tr>
<tr>
<td>ANA</td>
<td></td>
</tr>
<tr>
<td>Elevated anti-dsDNA antibody ($&gt; 100$ IU/ml)</td>
<td>35 (50.0)</td>
</tr>
<tr>
<td>Low serum C3 ($&lt; 76$ mg/dl)</td>
<td>42 (60.0)</td>
</tr>
<tr>
<td>Low serum C4 ($&lt; 9$ mg/dl)</td>
<td>17 (24.3)</td>
</tr>
<tr>
<td>Medications, $n$ (%)/mean (s.d.) (range)</td>
<td></td>
</tr>
<tr>
<td>Prednisolone/daily dose among users, mg</td>
<td>51 (72.9)/91.7 (7.6) (2–40)</td>
</tr>
<tr>
<td>HCO/daily dose among users, mg</td>
<td>36 (51.4)/228.6 (96.8) (200–400)</td>
</tr>
<tr>
<td>AZA/daily dose among users, mg</td>
<td>32 (45.7)/68.2 (26.0) (25–100)</td>
</tr>
<tr>
<td>Mycophenolate mofetil/daily dose among users, g</td>
<td>12 (17.1)/1.4 (0.5) (0.75–2.0)</td>
</tr>
<tr>
<td>Cyclophosphamide/daily dose among users, mg</td>
<td>3 (4.3)/100 (0)</td>
</tr>
</tbody>
</table>

Mo Yin Mok et al.
lowest detection limit of the assay (0.7 ng/ml). IL-33 was detected in three SLE patients (4.3%) compared with two control subjects (7.1%) \((P = 0.62)\). The levels of IL-33 between these SLE patients (1.23, 1.45 and 2.90 ng/ml) and controls (0.72 and 2.99 ng/ml) were not statistically significant \((P = 0.53)\). These patients did not demonstrate symptoms suggestive of allergic diseases or atopy at the time of study. On the other hand, SLE patients were found to have significantly higher serum levels of sST2 \([0.45 (0.14) \text{ ng/ml}]\) than controls \([0.36 (0.13) \text{ ng/ml}]\) \((P < 0.001)\).

Serum sST2 level and organ involvement
Serum sST2 level was not found to be related to gender \((P = 0.98)\) or age \((P = 0.66)\). Among the 127 samples collected for measurement of sST2, 35 samples were taken during active lupus nephritis, 27 during non-renal active lupus exacerbations and 65 samples when the patients had inactive disease. ANOVA analysis demonstrated a difference in serum sST2 levels between these three groups and normal controls \((P < 0.001)\). Figure 1 shows the serum levels of sST2 and IL-33 among active and inactive SLE patients and normal controls. Patients who had active lupus nephritis had significantly higher serum sST2 \([0.52 (0.21) \text{ ng/ml}]\) compared with patients with inactive disease \([0.42 (0.08) \text{ ng/ml}]\) \((P = 0.02)\) and with normal controls \((P < 0.001)\). Interestingly, ANOVA analysis showed a difference in sST2 level between patients who had Class III, Class IV and mixed Class IV and V lupus nephritis \((P = 0.02)\). Patients who had pure Class IV lupus nephritis \([0.63 (0.14) \text{ ng/ml}]\) had a higher serum sST2 level compared with those who had Class III \([0.33 (0.04) \text{ ng/ml}]\) and mixed Class IV and V lupus nephritis \([0.46 (0.04) \text{ ng/ml}]\) \((P = 0.006)\). Patients who had non-renal active exacerbations \([0.49 (0.12) \text{ ng/ml}]\) were also found to have higher serum sST2 compared with patients with inactive disease \((P = 0.03)\) and normal controls \((P < 0.001)\). However, serum sST2 levels were not significantly different between patients who had active lupus nephritis and those who had non-renal active disease \((P = 0.85)\). Serum sST2 levels were not particularly different in patients with and without cutaneous and articular \((P = 0.81)\) or haematological involvement \((P = 0.07)\). Twenty-nine patients had impaired eGFR at the time of study. Serum sST2 levels were not found to be different between patients who had impaired renal function \([0.44 (0.10) \text{ ng/ml}]\) and those who had normal renal function \([0.43 (0.17) \text{ ng/ml}]\) \((P = 0.36)\).

Serum sST2 level and medications
Serum level of sST2 was found to have a significant correlation with daily dosage of prednisolone \((r = 0.27; P = 0.002)\) but not other immunosuppressive drugs including HCQ \((P = 0.62)\), AZA \((P = 0.37)\), cyclophosphamide \((P = 0.52)\) or mycophenolate mofetil \((P = 0.36)\). Patients on prednisolone had higher serum sST2 level \([0.47 (0.14) \text{ ng/ml}]\) compared with those not taking the medication \([0.44 (0.15) \text{ ng/ml}]\) \((P = 0.03)\).
Correlation of IL-33 and sST2 with parameters of disease activity

Serum IL-33 level was not found to correlate with the level of sST2 in SLE patients \( r = 0.03; P = 0.80 \) or controls \( r = 0.07; P = 0.73 \). The SLEDAI score of the three SLE patients with elevated IL-33 were 0, 8, and 12, respectively. One of these patients had active lupus nephritis, one had non-renal active lupus whereas the third had inactive disease.

On the other hand, serum sST2 level at the time of active disease \( [0.51 (0.18) \, \text{ng/ml}] \) was significantly higher compared with serum samples taken during inactive disease \( [0.42 (0.08) \, \text{ng/ml}] \) \( P = 0.006 \) and with normal controls \( [0.36 (0.13) \, \text{ng/ml}] \) \( P < 0.001 \) (Fig. 2). SLE patients who had inactive disease were also found to have higher serum sST2 level than normal controls \( P < 0.001 \). Patients with elevated anti-dsDNA antibody (\( >100 \, \text{IU/ml} \)) had a higher level of sST2 \( [0.53 (0.17) \, \text{ng/ml}] \) than those who had lower anti-dsDNA antibody \( [0.44 (0.13) \, \text{ng/ml}] \) \( P = 0.001 \). Patients with low C3 level \( (<76 \, \text{mg/dl}) \) had a higher level of sST2 \( [0.49 (0.16) \, \text{ng/ml}] \) than those with normal C3 level \( [0.41 (0.08) \, \text{ng/ml}] \) \( P = 0.002 \). Similarly, patients with low C4 level \( (<9 \, \text{mg/dl}) \) were also found to have a higher level of sST2 \( [0.51 (0.14) \, \text{ng/ml}] \) than those with normal C4 level \( [0.45 (0.14) \, \text{ng/ml}] \) \( P = 0.01 \).

SLEDAI score was shown to correlate moderately with markers of disease activity, including serum levels of anti-dsDNA antibody \( r = 0.70; P < 0.001 \), C3 \( r = -0.76; P < 0.001 \), C4 \( r = -0.57; P < 0.001 \) and weakly with lymphocyte count \( r = -0.24; P = 0.006 \). Similarly, serum level of sST2 was found to correlate mildly with SLEDAI \( r = 0.30; P = 0.001 \) and serum level of anti-dsDNA antibody \( r = 0.32; P < 0.001 \) and negatively with C3 \( r = -0.32; P < 0.001 \) but not C4 \( r = -0.13; P = 0.13 \) or lymphocyte count \( r = -0.05; P = 0.59 \) (Fig. 2).

Sensitivity to change in serum sST2 level

Serum sST2 level was measured serially in 57 patients. Among these patients, 25 patients had active disease, whereas 32 had inactive disease at the time of the second measurement. These 57 patients included 9 patients who had decreased disease activity, 4 patients who had increased disease activity and 44 patients who had no change in the level of disease activity compared with the first assessment. Figure 3 shows the changes in serum sST2 level in relation to changes in level of disease activity between the first and second visits of longitudinal follow-up. The changes in serum sST2 level at the first and second evaluations among the 44 patients without serial change in level of disease activity was not found to be statistically different \( [0.46 (0.12) \, \text{vs} \, 0.48 (0.16) \, \text{ng/ml}] \) \( P = 0.34 \). However, the changes in serum sST2 levels were found to be significantly different for the 13 patients who demonstrated serial change in disease activity \( [0.53 (0.20) \, \text{and} \, 0.41 (0.09) \, \text{ng/ml for the occasions with higher and lower disease activity, respectively}] \) \( P = 0.02 \) with an effect size of sensitivity to change of \( \delta = 0.29 \).

Multiple logistic regression analysis

Univariate analysis revealed that serum sST2 level correlated with levels of anti-dsDNA antibody, C3 and daily prednisolone dosage. Multiple logistic regression analysis using disease status (with SLEDAI > 4 considered to be active) as dependent variable and serum sST2 level and daily prednisolone dosage as independent variables showed that both serum sST2 and prednisolone dosage were significant predictors of active lupus. Prednisolone dosage was found to contribute to active lupus with an OR of 1.12 (95% CI 1.03, 1.22; \( P = 0.008 \)), whereas serum sST2 remained significantly associated with active SLE disease after adjustment for daily prednisolone dosage (OR 4.6; 95% CI 2.7, 3601.0; \( P = 0.01 \)). As SLEDAI is a composite score involving components including anti-dsDNA antibody, C3 and C4, linear regression analysis was performed using a modified SLEDAI score that excluded elevated anti-dsDNA antibody, low C3 and C4 as dependent variable. Other than increased anti-dsDNA antibodies (regression coefficient 2.2; 95% CI 1.3, 3.0; \( P < 0.001 \)), serum sST2 level was also found to be an independent predictive factor for modified SLEDAI (regression coefficient: 8.5; 95% CI 2.6, 14.3; \( P = 0.005 \)) by stepwise regression analysis after controlling for age, sex, eGFR and daily prednisolone dosage. In other words, the modified SLEDAI score was found to increase by an average of 0.85 U for each 0.1 ng/ml increment in serum sST2 level.

Discussion

Our study is the first to reveal an elevated serum level of sST2 in SLE patients in correlation with disease activity. Although serum sST2 level has also previously been demonstrated to be higher in SLE patients in accordance with our finding, as well as in other rheumatic diseases compared with normal controls [22], information with regard to its clinical association is lacking. We demonstrated the higher sST2 levels among SLE patients with active disease including renal and non-renal manifestations compared with those with lesser disease activity and normal controls. In view of the significant correlation between serum sST2 level and parameters of disease activity, its responsiveness to change in the levels of disease activity when monitored longitudinally and its lack of association with age, sex and impaired renal function, sST2 may serve as a potential surrogate marker for disease activity in SLE.

Serum sST2 level was found to correlate with SLEDAI, levels of anti-dsDNA antibody and C3. Serum sST2 levels remained significantly associated with active disease after adjustment for prednisolone dosage in the logistic regression model, eliminating contribution from prednisolone use as a confounder for active lupus. Since the biochemical properties of sST2 in relation to age, sex and renal clearance is not known, a modified SLEDAI score as dependent variable that excluded elevated anti-dsDNA antibody, low C3 and C4, was used in our analysis, further confirming both anti-dsDNA antibody as a conventional
biomarker and sST2 significant predictive factors of disease activity after controlling for age, sex, eGFR and daily prednisolone dosage. The elevated serum sST2 level found in patients with active disease was, however, not shown to be discriminative between active lupus nephritis and non-renal lupus exacerbation. Serum sST2 was not found to be linked to particular disease manifestations such as cutaneous and articular or haematological involvement and thus appeared to be a marker of generalized disease activity in SLE patients. It should be noted that serum sST2 level was higher among Class IV lupus nephritis compared with other ISN/RPS classes, suggesting a reflection of a higher level of inflammation by the higher level of sST2. Other than SLE, elevated serum level of sST2 has also been described in other diseases including septic shock [13], asthma [14] and myocardial infarction [15]. sST2 was also found to correlate positively with disease activity and severity in these medical conditions as do peak flow rate in acute asthma exacerbation and creatinine kinase in patients with myocardial infarction, suggesting its role as a useful biomarker in these conditions [28]. Serum sST2 has also been reported to be of prognostic value in the prediction of cardiac mortality in patients with acute myocardial infarction [29] and acute destabilized heart failure [30]. As we can only demonstrate clinical association instead of causation, further studies are required to delineate the role of sST2 as merely a biomarker in lupus, or as a regulatory mechanism involved in the disease pathogenesis.

Fig. 2 Correlation between serum sST2 level and parameters of disease activity. (A) SLEDAI, (B) serum C3 level, (C) anti-dsDNA antibody level and (D) lymphocyte count.

![Fig. 2](image)

**Fig. 3** Changes in serum sST2 level in relation to changes in level of disease activity between the first and second visits of longitudinal follow-up on SLE patients.
Mo Yin Mok et al.

Beyond its role as a biomarker, sST2 may also be directly involved in the regulation of immune-mediated conditions. The expression of ST2L in haematopoietic cell lines and its homology with members of the TLR-IL-1R family suggest its potential functional role in immune responses [31]. Indeed, sST2 has been shown to inhibit pro-inflammatory cytokine production. Lipopolysaccharide (LPS)-induced IL-6 production in a human monocytic cell line was suppressed in the presence of sST2 via inhibition of NF-κB activation [32]. Administration of sST2 to mice exposed to LPS in a model of sepsis has been shown to result in reduced serum levels of IL-6, -12 and TNF-α and enhanced survival of the animal [33]. The present findings showing an inverted correlation between sST2 and C3 levels in lupus patients indicate, therefore, the potential regulatory role of sST2 in the disease.

Our study is also the first to report serum levels of the sST2-specific ligand, IL-33, in SLE patients. Elevated IL-33 was infrequently observed in SLE patients and was found to be comparable in frequency and serum level to control although our conclusion may be limited by the sensitivity of the immunoassay. Serum IL-33 level was not found to be related to sST2 level, lupus disease activity or specific organ involvement. Thus, the role of sST2 in relation to IL-33 signalling in SLE remains unclear. It is possible that IL-33 may not be involved in the pathogenesis of SLE or that IL-33 may have formed ICs with sST2 or be down-regulated by sST2 in the negative regulatory mechanisms. Indeed, sST2 has recently been demonstrated to function as a negative regulator and antagonistic decoy receptor for IL-33 [34, 35].

We have not determined the source of IL-33 and sST2 in lupus patients in this study. A diversity of cell types have been reported to express IL-33 mRNA including smooth muscle cells, epithelial cells, fibroblasts, keratinocytes, dendritic cells and activated macrophages [1]. IL-33 was induced in TNF-α- and IL-1-activated fibroblasts and keratinocytes, whereas expression of IL-33 mRNA was only modestly elevated in LPS-stimulated dendritic cells and macrophages [1]. Expression of sST2 was also induced in fibroblasts, macrophages and monocytes stimulated with LPS, TNF-α or IL-1 [31]. It has been speculated that IL-33 may be released as a result of cell damage, which alerts the immune system to ‘danger’, but otherwise remains in the nucleus of living cells where it limits the immune response [36]. In pathological conditions, abundant expression of IL-33 mRNA has been found in endothelial cells from inflamed human tonsil, intestine of patients with Crohn’s disease and rheumatoid synovium [37]. Thus, it is possible that sST2 and/or IL-33 are released from activated macrophages, dendritic cells or endothelial cells from inflamed tissues in patients with active SLE.

In accordance with the recent discovery of the role of the IL-17/IL-23 axis in many pathogenic inflammatory conditions [38], understanding the functional aspect of the IL-33/sST2 system may have significant therapeutic implications. Indeed, there have been increasing reports on manipulating the IL-33/sST2 system in the alleviation of disease conditions. Pre-treatment with sST2 has been shown to significantly reduce eosinophilic infiltration and levels of IL-4 and -5 in bronchoalveolar lavage fluid in a murine model of allergic airway inflammation [39]. A soluble ST2–human IgG fusion protein has also been shown to be of therapeutic effect in animal models including attenuation of eosinophilic inflammation in a model with hyper-responsiveness [40] and in vitro experiments to inhibit TNF-α production by a human monocytic cell line in co-culture with peripheral blood T cells from patients with RA [40]. Our findings of elevated serum levels of sST2 and the correlation with disease activity suggested that the IL-33/sST2 system may have a role in the pathogenesis of SLE. Future efforts to define the regulatory mechanisms of the system may hopefully result in more therapeutic options for these patients.

Rheumatology key messages

- Soluble ST2 is elevated in SLE patients compared with healthy controls.
- Serum sST2 level correlates with parameters of lupus disease activity.
- The level of IL-33, its specific ligand, is only infrequently detected in SLE serum by ELISA.

Acknowledgements

We would like to thank Miss Wu Haijing for help with the graphics in the paper.

Funding: This research is supported by a University Research grant from the University of Hong Kong.

Disclosure statement: The authors have declared no conflicts of interest.

References

1 Schmitz J, Owyang A, Oldham E et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005;23:479–90.
7 Moulin D, Donze O, Talabot-Ayer D, Mezin F, Palmer G, Gabay C. Interleukin (IL)-33 induces the release of
32 Takezako N, Hayakawa M, Hayakawa H et al. ST2 suppresses IL-6 production via the inhibition of IkappaB degradation induced by the LPS signal in THP-1 cells. Biochem Biophys Res Commun 2006;341:425–32.
34 Dinarello CA. An IL-1 family member requires caspase-1 processing and signals through the ST2 receptor. Immunity 2005;23:461–2.
37 Carriere V, Roussel L, Ortega N et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. Proc Natl Acad Sci USA 2007;104:282–7.