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Elevated IL-33 and sST2 levels in sera of patients with pemphigus vulgaris. Correlation with clinical and immunological parameters during follow up.

Running head: IL-33 and sST2 in pemphigus vulgaris.

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Pemphigus vulgaris (PV), an autoimmune-mediated blistering disease in the skin and mucous membranes, is mediated by antidesmoglein 1 and/or 3 autoantibodies leading to loss of cohesion between the keratinocytes in the epidermis.¹ The exact molecular pathogenesis of the disease remains unclear,

however, it seems that dysregulation of cytokine signaling may contribute to PV.

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Interleukin-33 (IL-33) and soluble ST2 (sST2), members of the IL-1 family, are predominantly found in the skin, lung, adipocytes, and synovial fibroblasts.^{3,4}

Both cytokines seem to have a considerable role in regulation of inflammation, whereas excessive production contributes to the pathogenesis of autoimmune diseases.⁵

The aim of the present study was to assess the potential role of IL-33 and sST2 in PV, and to explore their possible relation with clinical features and immunological parameters.

Sixty new and consecutive PV patients (with active disease, who were treatment naïve or with relapse) and 30 healthy age/sex matched, unrelated individuals entered to the study. All patients gave written informed consent to the procedures. All patients underwent blood sampling and IL-33 and sST2 serum levels were measured by the ELISA technique (antibodies-online, Inc, USA).

Disease severity was determined according to the Pemphigus Vulgaris Activity Score (PVAS).⁶ The patients were arbitrarily categorized into two groups: 1. Patients with mild disease: 6/18 points of PVAS, and 2. Patients with severe disease: >13/18 points of PVAS. To make a reliable conclusion on the relationship between the severity of the disease and the serum levels of cytokines, patients with moderate severity (7-12/18 points of PVAS) were excluded from the study.

Table 1 shows that IL-33 and sST2 levels in PV patients were significantly higher than healthy controls ($P = .001$ for both cytokines).

After one month of therapy, IL-33 and sST2 levels significantly decreased in all patients (Fig 1); furthermore, 45 patients had decreased disease activity and 15 patients had moderate change compared with the first assessment. Significant changes in sST2 and IL-33 levels at the first and second evaluations among the 15 patients with moderate changes in disease severity were observed (sST2: 79.61 ± 15.79 vs 49.69 ± 3.87 pg/mL, $P = .001$; IL-33: 625.42 ± 94.54 vs 429.07 ± 42.95 pg/mL, $P = .001$). Similarly occurred in patients with marked changes in disease severity (IL-33: 462.49 ± 116.3 vs 264.7 ± 102.23 pg/mL, $P = .001$; sST2: 71.08 ± 16.32 vs 39.79 ± 6.21 pg/mL, $P = .001$).

IL-33 and sST2 levels correlated with daily prednisone dosage ($r = 0.31$; $P = .001$ and $r = 0.43$, $P = .001$, respectively). Patients with prednisone 1mg/kg/day, had higher IL-33 and sST2 levels (585.32 ± 92.3 and 84.56 ± 12.14 pg/mL, respectively) compared with those with <1mg/kg/day (433.21 ± 87.6 and 53.21 ± 12.15 pg/ml, respectively).

Patients with elevated anti-Dsg3 antibody (>150 IU/mL) had higher sST2 (75.98 ± 15.35 pg/mL) and IL33 levels (533.73 ± 118.55 pg/mL) than those with lower anti-Dsg3 antibody (sST2: 62.14 ± 16.84 pg/mL, $P = .02$; IL-33: 381.2 ± 110.82 pg/mL, $P = .001$).

Regression analysis using disease severity (with PVAS >13 or severe disease) as dependent variable and sST2 and IL-33 as independent variables, after controlling for age, sex, anti-Dsg3 and daily prednisone dosage, showed that

both sST2 and IL-33 were significant predictors of disease severity (IL-33: OR= 1.85, CI^{95%} 1.23 - 2.18; $P= .005$; sST2: OR= 3.3; CI^{95%} 2.1 – 5.18; $P= .001$).

Elevated serum levels of sST2 and IL-33 in PV patients have not previously reported. The exact mechanism of those cytokines in the pathogenesis of PV is unknown however, we hypothesized that IL-33 through inducing the production of IL-6, TNF α and promote the activation of Th2 cells (known mediators in the pathogenesis of PV), ⁷ can influence on disease activity.

We demonstrated a significant correlation between these cytokines and parameters of disease activity (PVAS, anti-Dsg3 antibodies), their responsiveness to change in the levels of disease activity when monitored longitudinally and the lack of association with age, sex and daily prednisone dosage, therefore, IL-33 and sST2 may serve as a potential surrogate marker for disease activity in PV.

Both cytokine levels remained significantly associated with severe disease after adjustment for corticosteroids dosage in the logistic regression model, eliminating contribution from prednisone use as a confounder. Since the biochemical properties of IL-33 and sST2 in relation to age or sex are not known, a PVAS score as dependent variable that excluded elevated anti-Dsg3 antibody, was used in our analysis, further confirming both anti-Dsg3 antibody as a conventional biomarker and IL-33 and sST2 significant predictive factors of severe disease after controlling for age, sex and daily prednisone dosage. It should be noted that both IL-33 and sST2 levels were higher among patients with more extensive cutaneous area affected compared with mild extension,

suggesting a reflection of a higher level of inflammation by the higher level of IL-33 and sST2.

Elevated sST2 levels have been described in septic shock,⁸ however, the patients in our study had no data suggestive of severe sepsis or septic shock, indicating that disease activity is independently associated with both cytokine levels.

Hence, these data suggest that IL-33 and sST2 may contribute to the pathogenesis of PV and could be considered markers of disease activity. Our data are in accordance with previous studies in RA, ankylosing spondylitis, and Henoch-Schönlein purpura, showing that serum IL-33 levels are correlated with disease activity markers.⁹⁻¹¹

In terms of limitations, we could not determine the cause of the elevated IL-33 and sST2 levels, however IL-33 is released from skin structural cells by several trigger factors including infectious agents, mechanical injury and allergens, and acts on several STD-expressing cells such eosinophils and Th2 cells and therefore Th2-associated cytokines (mainly IL-5 and IL-13).¹² Moreover, as we can only demonstrate clinical association instead of causation, further studies are required to delineate the role of sST2/IL33 as merely a biomarker in pemphigus, or as a regulatory mechanism involved in the disease pathogenesis. Future efforts to determine the regulatory role of these cytokines are required, which will help to improve the therapeutic options for these patients.

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Tables.

Table 1. Clinical, demographic and laboratory data.

Variable	Mild PV (n= 29)	Severe PV (n= 31)	Healthy controls (n= 30)
Age (yr), mean $\pm$ SD	46.8 $\pm$ 13.2	45.1 $\pm$ 12.8	45.2 $\pm$ 14.23
	Total: 45.3 $\pm$ 13.47		
Sex ratio (M/F)	13/16	14/16	14/16

IL-33 (pg/mL) ± SD	439.46 ± 115.98	562.87 ± 117.49	155.82 ± 31.85
	Total: 503.22 ± 131.42 *		
sST2 (pg/mL) ± SD	66.07 ± 16.41	79.9 ± 13.69	30.67 ± 7.79
	73.21 ± 16.48 *		
Disease duration (months), mean ± SD	5.74 ± 2.25	5.55 ± 2.18	ND
	Total: 5.68 ± 4.34		
Anti-dsg 3 (UI/mL) ± SD	158.33 ± 31	255.75 ± 37.32	ND
	208.66 ± 59.78		

PV = Pemphigus vulgaris; ND = Not determined. * Statistically significant compared with controls.

Figures.

Fig 1. Comparison between serum sST2 and IL-33 levels at baseline and four weeks after treatment.

