Development of a highly sensitive assay to quantitate circulating thymic stromal lymphopoietin (TSLP) levels in blood

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Introduction

- Thymic stromal lymphopoietin (TSLP) is an epithelial cytokine, released in response to cellular damage or inflammation, which activates multiple cell types and downstream inflammatory pathways associated with asthma and other allergic diseases.[1,2]
- Tezepelumab is a human monoclonal antibody that specifically blocks TSLP from interacting with its receptor complex and is in development for the treatment of severe, uncontrolled asthma[3,4] and atopic dermatitis (AD).
- Levels of TSLP are upregulated in the airways of patients with asthma and correlate with expression levels of type 2 T-helper cell-attracting chemokines, levels of type 2 innate lymphoid cells and disease severity.[5,6]
- Levels of TSLP have been reported to be increased in keratinocytes from skin lesions of patients with AD.[6]
- In some disease settings, localized, episodic release and rapid turnover of TSLP may result in low circulating concentrations of TSLP.
- Existing immunoassays that detect TSLP may not have the sensitivity required to accurately quantify circulating TSLP.
- We report the development of a highly sensitive assay for the quantification of soluble TSLP, which may have utility in clinical trials with tezepelumab.

Methods

- An electrochemiluminescence fit-for-purpose S-PLEX assay (Meso Scale Discovery [MSD], Gaithersburg, MD, USA) was developed to quantify serum levels of TSLP with an improved lower limit of quantification (LLOQ) and lower limit of detection (LLOD) than commercially available immunoassays.
- Inter-assay and intra-assay reproducibility were assessed.
- The novel assay was used to quantify serum levels of TSLP from patients with AD and healthy individuals without atopic disease in a non-interventional, Institutional Review Board-approved study.
  - AD severity status was determined by the Eczema Area and Severity Index (EASI) score.[7]
  - Swabs from both lesional and non-lesional skin samples were obtained from patients with AD (two samples per patient) and were cultured for detection of colonization with *Staphylococcus aureus*.
  - Healthy individuals without atopic disease were identified through quantification of total and allergen-specific immunoglobulin E levels.
- Mann–Whitney tests were used to obtain *p* values for comparisons between healthy individuals and patients with AD, and between patients with AD who were positive or negative for *S. aureus* colonization.

Table 1. Available assays measuring circulating TSLP

Parameter	In development	Commercially available			
	S-PLEX assay (MSD)	S-PLEX assay (MSD)	V-PLEX assay (MSD)	U-PLEX assay (MSD)	Quantikine assay (R&D Systems)
LLOQ, fg/mL	12.0	60.0	460.0	Not provided	Not provided
LLOD, fg/mL	1.3	1.5	63.0	200.0	9870.0
Range of detection, fg/mL	12–22,828	60–20,000	300–2000	200–10,100,000	31,200–2,000,000

LLOD, lower limit of detection; LLOQ, lower limit of quantification; MSD, Meso Scale Discovery; TSLP, thymic stromal lymphopoietin.

Results

Novel S-PLEX assay parameters

- The technically validated LLOQ for the novel S-PLEX assay was 12.0 fg/mL, which is substantially lower than the LLOQ of commercially available assays (60.0–460.0 fg/mL) (**Table 1**).
- The LLOD for the novel S-PLEX assay (1.3 fg/mL) was also lower than that of commercially available assays (1.5–9870.0 fg/mL) (**Table 1**).
- The range of detection of the novel S-PLEX assay was 12–22,828 fg/mL (**Table 1**).
- The inter-assay and intra-assay coefficients of variation were 20% or less.

Serum levels of TSLP in healthy individuals without atopic disease and patients with AD

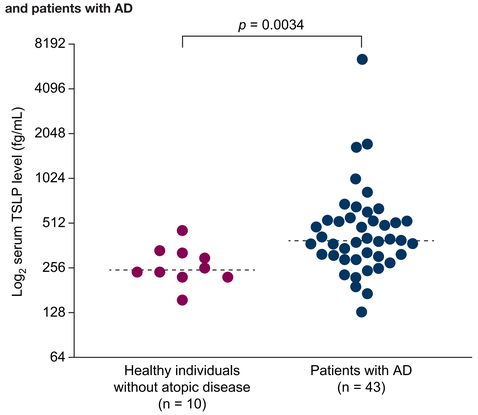
- Overall, 43 patients with AD (mild-to-moderate AD, n = 26; severe-to-very-severe AD, n = 17) and 10 healthy individuals were included in the study (**Table 2**).
  - Among patients with AD, the median (range) EASI score was 16.8 (5.8–69.0).
- Serum levels of TSLP were significantly higher in patients with AD than in healthy individuals ($p = 0.0034$) (**Figure 1**).
  - The median (range) serum levels of TSLP were 397 (131–6514) fg/mL and 252 (158–461) fg/mL in patients with AD and healthy individuals, respectively.
- Patients with severe-to-very-severe AD had higher serum levels of TSLP than those with mild-to-moderate AD (median [range], 502 [249–1741] fg/mL vs 376 [131–6514] fg/mL; $p = 0.0610$) (**Figure 2**).
- Patients with mild-to-moderate AD and severe-to-very-severe AD had significantly higher median levels of TSLP than healthy individuals ($p = 0.0364$ and $p = 0.0004$, respectively) (**Figure 2**).
- Patients with AD who were positive for *S. aureus* colonization had significantly higher median levels of TSLP than those who were negative for *S. aureus* colonization and than healthy individuals ($p = 0.0252$ and $p = 0.0003$, respectively) (**Figure 3**).

Table 2. Demographics and clinical characteristics of patients with AD and healthy individuals without atopic disease

Characteristic	Patients with AD (n = 43)	Healthy individuals without atopic disease (n = 10)
Age, years	35 [20–63]	41 [26–59]
Men, n (%)	22 (51)	4 (40)
Ethnicity, n (%)		
Caucasian	24 (56)	6 (60)
African American	7 (16)	1 (10)
Other	12 (28)	3 (30)
Mild-to-moderate AD, n (%)[a]	26 (60)	–
Severe-to-very-severe AD, n (%)[a]	17 (40)	–
Rajka and Langeland eczema severity score	8 [6–9]	–
EASI score	16.8 [5.8–69.0]	–
Blood eosinophils, %	2.9 [1.0–8.6]	1.2 [0.5–3.2]
Blood eosinophils, cells/μL	200 [70–680]	100 [30–950]
Total IgE, kIU/L	162 [2–20,976]	10 [2–67]
ImmunoCAP IgE, kUA/L	9.5 [0.1–906.0]	0.1 [0.1–4.2]
S. aureus status, lesional skin (positive/negative)[b]	20[c]/23	–
S. aureus status, non-lesional skin (positive/negative)[b]	14[c]/29	–

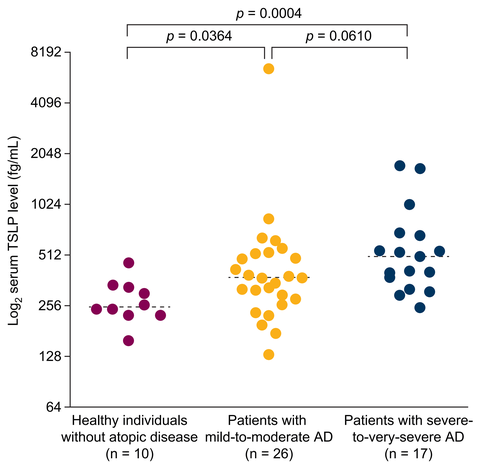
Data are median [range] unless stated otherwise.
[a]AD severity status was determined by the EASI (mild-to-moderate AD, < 21.0; severe-to-very-severe AD, ≥ 21.0). [b]Two skin samples were obtained from each patient with AD. [c]Of samples positive for *S. aureus* colonization, MRSA was detected in three lesional skin samples and no non-lesional skin samples.
AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IgE, immunoglobulin E; IU, international units; MRSA, meticillin-resistant *S. aureus*; UA, units of allergen.

Figure 1. Serum levels of TSLP in healthy individuals without atopic disease and patients with AD



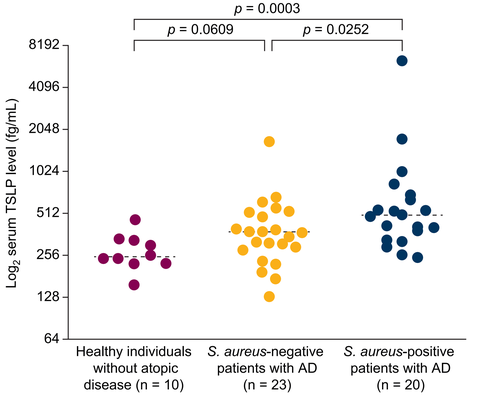
AD, atopic dermatitis; TSLP, thymic stromal lymphopoietin.

Figure 2. Serum levels of TSLP in healthy individuals without atopic disease and patients with mild-to-moderate AD or severe-to-very-severe AD



AD, atopic dermatitis; TSLP, thymic stromal lymphopoietin.

Figure 3. Serum levels of TSLP in healthy individuals without atopic disease and patients with AD who were negative or positive for *S. aureus* colonization



AD, atopic dermatitis; TSLP, thymic stromal lymphopoietin.

Conclusions

- The novel S-PLEX assay had a higher sensitivity and a better LLOQ than commercially available assays and may be useful for evaluating TSLP levels in clinical studies with tezepelumab.
- The novel S-PLEX assay was able to detect TSLP in all samples tested, levels of which were lower than those detected using commercially available assays.
- Higher serum levels of TSLP were detected in patients with AD than in healthy individuals and appeared to be associated with greater AD severity.
- Previously, *S. aureus* colonization has been shown to be correlated with AD severity.[8] In the present study, patients with AD who were positive for *S. aureus* colonization had higher levels of TSLP than those who were negative for *S. aureus* colonization, and than healthy individuals. These findings suggest that *S. aureus* colonization may contribute to the elevated serum levels of TSLP observed in patients with AD.[9,10]

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Disclosures

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The product mentioned is investigational and not approved in any country.

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