Prurigo nodularis blood signature is characterized by increases in IL-13 and caspase 8

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Support provided by Eli Lilly and Company

Conflict of Interest Disclosures: Gil Yosipovitch has served as an advisory board member, investigator and/or received consulting fees from Galderma, Pfizer, Sanofi Regeneron, Novartis, Eli Lilly, Kiniksa, Cellu Therapeutics and Cell Dex. Sarah Engle, Autum Auxier, Sean Sissons, Jonathan Sims, Zhe Sun and Angela Okragly are all employees and potentially stockholders of Eli Lilly. All other authors state no additional conflicts of interest to disclose.
**INTRODUCTION**

- Prurigo nodularis (PN) is an inflammatory skin condition characterized by severely pruritic, hyperkeratotic nodules distributed symmetrically across the trunk and extremities.
- It is extremely burdensome, with an estimated prevalence of 36.7-43.9 per 100,000 population.
- Immune dysregulation is suggested to underly the mechanism of itch in PN.
- The type 2 immune response plays a key role with interleukin (IL)-4, IL-13, IL-31 and periostin elevated in PN lesional skin. Helper T cell (Th)17 and Th22 related genes and cytokines are also upregulated in PN tissue.
- Olink is the most comprehensive inflammatory panel to date that can simultaneously analyze 92 protein biomarkers.
- Recent proteomic studies utilizing Olink have found inflammatory serum biomarkers in atopic dermatitis and hidradenitis suppurativa that correlate with clinical severity and/or therapeutic responses.
- To our knowledge, no such similar study on PN have been published.

**OBJECTIVES**

1. Characterize the systemic inflammatory signature of PN.
2. Correlate proteomic signature to patient itch severity scores.

**METHODS**

**Human subjects:**
- Serum samples collected from 33 patients with PN and 20 age and sex matched healthy controls (HC).
- Exclusion criteria: concomitant atopic diagnosis.
- Demographics, Investigator Global Assessment (IGA), duration of itch, and average 24-hour numerical rating scale (NRS) for self-reported itch intensity were collected.

**Immuonoassays:**
- Olink Target 96 Inflammation Panel.
- Single-plex immunoassay:
  - IL-13 (Simoa® Human IL-13 Advantage HD-1/HD-X kit).
  - IL-17A (Simoa® Human IL-17A HD-1/HD-X 2.0 kit).
  - IL-4 (MSD S-PLEX Human IL-4 kit).
  - IL-5 (MSD S-PLEX Human IL-5 kit).
  - Periostin (Invitrogen Human periostin ELISA kit).
  - IgE (Invitrogen Human IgE ELISA kit).
  - IL-22 MSD immunoassay (Lilly in-house assay).

**Statistical Analysis:**
- One-way ANOVA to compare PN patients and HC using log-transformed Olink proteomic data and single-plex data.
- For between-markers multiplicity adjustment, q-value was calculated with a Benjamini-Hochberg procedure with the significance threshold at 0.05.
- Spearman’s correlation was utilized to find relationships between protein levels and clinical data.
**RESULTS: PROTEOMIC ASSAYS**

- **Patient Clinical Data**
  - IGA ≥ 3
  - NRS: 7.67 ± 0.4 [0-10]*
  - Duration of itch: 10.3 ± 1.5 [1-30]* years
*Values expressed by mean ± SEM [range]

- Thirteen proteins with missing data frequency greater than 80% were removed from analysis, e.g. IL-13, IL-5, etc.

- Of the remaining proteins and using 1.3-fold changes (FC) as the minimum threshold to ensure biological relevance:
  - **Caspase 8 (CASP8)** (FC = 1.56, p = 9.35E-5) was significantly elevated
  - **IL-17C** (FC = -2.61, p = 1.38E-9) was significantly decreased
  - No other signals of fibrosis or pro-fibrosis were detected
  - **IL13_S** from single-plex assay. IL-13 from Olink panel did not yield enough data frequency to be evaluated and removed.

Volcano plot of proteomic assays showing analytes with >1.2-fold changes that were significant (qval < 0.05). Blue circles represent analytes significantly reduced in PN vs HC. Red circles represent analytes significantly elevated in PN vs HC. “S” indicates single-plex assay. Protein markers with missing data frequency >80% were removed from Olink data analysis (IL20RA, IL2RB, IL1 alpha, IL2, IL22RA1, Beta-NGF, IL13, ARTN, IL20, IL33, LIF, NRTN, IL5). FLT3L: fms-related tyrosine kinase 3 ligand. IL13_S: Interleukin-13 from single-plex analysis. CASP8: Caspase 8. TRAIL: tumor necrosis factor-related apoptosis-inducing ligand. IL17C: interleukin-17 C.
RESULTS: ULTRASENSITIVE SINGLE-PLEX DATA

- Significant **elevation** in **IL-13** (FC = 4.39, p= 0.0004)

- Other biomarkers IL-4, IL-5, IL-17A, IL-22, periostin, and IgE did not yield significant results

- IL-31, a common pruritogen found in the lesional skin of PN patients, was not evaluated due to limitations in the sensitivity and reliability of current available immunoassays

- Average NRS itch scores did not correlate (cor < 0.3) with IL-13 or CASP8
  - Likely due to relatively high itch intensity observed with this patient cohort

Analysis of IL-13, IL-4, IL-5, IL-17A, IL-22, periostin, and IgE by single-plex immunoassay
DISCUSSION

- Serum proteomic signature of PN showed significant elevation of IL-13, a key Th2 cytokine, providing further support that it has a significant role in PN.
- Although there was no demonstration of correlation with itch severity.

- Although CASP8 has been generally regarded to have anti-inflammatory properties, there are findings that suggest CASP8 may also be proinflammatory by inducing cytokine and chemokine expression depending on cell type and context. As CASP8 was upregulated in this study, it may play a more proinflammatory role in PN pathogenesis.

- Interestingly, IL-17C, which is upregulated in lesional PN, was downregulated in the serum.
- IL-17C is mainly expressed by epithelial cells rather than immune cells, which may explain this phenomenon. Other autoimmune inflammatory skin diseases have also shown either no significant difference or decreased levels of serum IL-17.

- Further studies are needed to elucidate the inflammatory and itch pathways related to PN and to evaluate the roles of the identified serum cytokines in PN disease severity.

REFERENCES