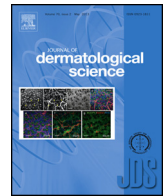




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Letter to the editor

Histamine, TNF, C5a, IL-6, -9, -18, -31, -33, TSLP, Neopterin, and VEGF are not elevated in chronic spontaneous urticaria

Spontaneous urticaria is a common skin disorder which is classified as acute or chronic depending upon disease duration (less or more than 6 weeks, respectively). At any one time, 0.5–1% of the population is thought to be suffering from chronic spontaneous urticaria (CSU). CSU is debilitating and results in a large socio-economic burden due to a reduction in performance of 20–30% [1]. The underlying cause of CSU is rarely identified, therefore, symptomatic treatment is the most frequently used form of management [1,2]. Treatment aims to ameliorate or suppress symptoms by inhibiting the release and/or the effect of mast cell mediators [2]. There are only a few differential diagnoses to CSU, including Schnitzler syndrome, urticaria vasculitis and Muckle Wells syndrome in patients with wheals, and ACE-inhibitor-induced or hereditary angioedema in patients with angioedema. As such, the diagnosis of CSU is not difficult with a detailed medical history and clinical examination, and does not require a surrogate marker. However, the objective assessment and monitoring of disease activity of CSU remains a challenge, and at present there are no prognostic markers identifying those patients with severe and long-lasting CSU. As a consequence, symptomatic therapy largely follows a 'trial and error' approach frequently involving multiple treatment adjustments with disease activity monitored using the urticaria activity score (UAS) [3].

Previously, several publications have claimed the identification of potential biomarkers of CSU, including various pro-inflammatory cytokines, growth factors and other mediators (Table 1). Most of them, however, were found to not correlate with disease severity or prognosis and have not been confirmed by independent investigators (Table 1). As a result, none of them have been established as a reliable marker of disease activity, possibly due to the limitations of this type of evaluation (e.g. number of subjects evaluated, difficulties in comparing data and measuring disease activity and impact between different centres). As such, there is an unmet need for biomarkers of urticaria to aid in diagnosis, prognosis and monitoring disease progression. The objective of

this study was to screen a large patient population for serum or plasma levels of previously reported or novel potential biomarkers. We chose to investigate either mediators that have been proposed before (Table 1) but have as of yet not been confirmed by others (TNF, IL-6, IL-9, IL-18, IL-31, Neopterin, VEGF), or mediators that have not been tested in urticaria patients before but which we hypothesized could be associated with urticaria activity because of their ability to activate mast cells or to be released by mast cells (histamine, C5a, IL-33, TSLP).

Blood samples were obtained from patients who had undergone the CSU diagnostic programme at Charité. Patients were selected randomly based on their UAS score (1–15 mild; 16–29 moderate; ≥30 severe) [3]. The validated Dermatology Quality-of-Life Index (DLQI) questionnaire was used to evaluate quality-of-life (QoL). Serum concentrations of potential biomarkers were measured by enzyme-linked immunosorbent assay (ELISA). Commercial kits were used to evaluate thymic stromal lymphopoietin (TSLP), VEGF, C5a, TNF, IL-6, IL-33, IL-31, IL-18 (R&D Systems Inc., Minneapolis, MN, USA), neopterin (DRG-Systems, Marburg, Germany), IL-9 (Biolegend, San Diego, California, USA) and histamine using the Histareader (RefLab ApS, Copenhagen, Denmark). All biomarkers were measured in sera, excluding C5a which was measured in plasma. All assays were performed according to the manufacturers' protocols. Potential biomarkers in plasma and/or sera were initially analysed in 28–30 patients with CSU with subsets of patients comprising of at least 9 patients from each UAS subgroup (mild, moderate, severe) and compared to 10 healthy controls. If the results of these analyses indicated a potential but statistically insignificant difference, we increased the number of plasma or sera analysed to 56–62 CSU patients (with a minimum of 16 patients from each subgroup). Statistical analysis of the relationship between disease severity and biomarker levels was carried out using ANOVA, with $p < 0.05$ considered statistically significant. The data were analysed using GraphPad Prism 5.

In order to confirm our definitions of mild, moderate, and severe disease based on the UAS7, QoL was measured using the DLQI questionnaire. Results from a total of 328 patients (164 mild, 131 moderate and 33 severe) were compared. Significant impairments in QoL were observed in both the moderate and severe subgroups

Table 1
Previously reported markers in chronic spontaneous urticaria.

Measured marker	Number of patients vs. controls	Number of centers	Correlation with disease activity	Ref.
IL-9	~50 vs. 48	1	No	[6]
IL-18	55 vs. 20	1	No	[7]
	73 vs. 40	1	No	[8]
IL-31	46 vs. 28	1	Not analyzed	[9]
MMP-9	29 vs. 18	1	No	[10]
	52 vs. 30	1	Yes	[11]
Neopterin	28 vs. 96	1	No	[12]
TNF, IL-6, IL-13, IL-12p70, IL-10	29 vs. 33	1	Not analyzed	[4]
VEGF	80 vs. 53	2	Yes	[13]

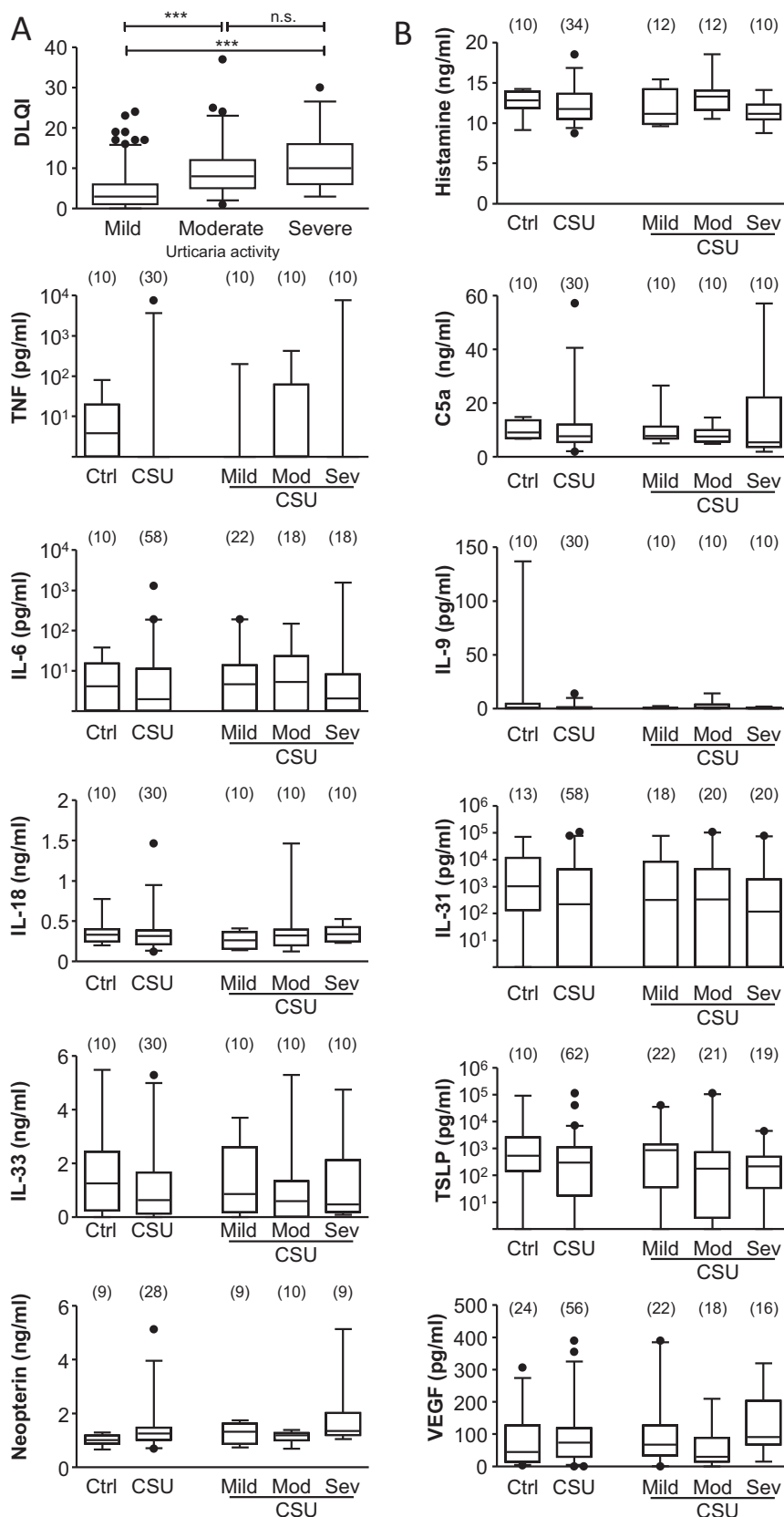


Fig. 1. (A) Differences in Dermatology Life Quality Index (DLQI) in different disease severity subgroups as defined by urticaria activity score (UAS): mild ($n = 164$) = UAS 1–15, moderate ($n = 131$) = UAS 16–29, severe ($n = 33$) = 30–42 and (B) sera concentrations of different possible disease mediators in healthy controls (Ctrl) and patients with chronic spontaneous urticaria (CSU). Different numbers of samples were analysed, number of patients per group is given in parentheses. All data are shown with median and lower and upper quartile, whiskers represent the 5th and the 95th percentile.

compared with the mild subgroup (Fig. 1A). No significant difference was observed in QoL when comparing the moderate and severe subgroups.

In the comprehensive ELISA analyses we were unable to identify significant correlation between any of the biomarkers evaluated and disease activity as assessed by the UAS7 (Fig. 1B).

In this study, we aimed to confirm and better characterize CSU biomarkers reported in the literature and to identify additional biomarkers of CSU disease activity. However, no direct correlations were found between any of the mediators examined and disease severity. This apparent discrepancy between our results and those previously published may be explained by a number of different factors, including the wide variability in the patient population from different centres and in different countries. The disease itself may also contribute to the discrepancy between studies, as the cause of CSU is rarely identified and the pathogenic mechanisms underlying CSU are largely unknown [1,2,4]. Thus, patient populations may have a wide pathological basis of CSU.

Reliable biomarkers can be utilized in many ways, for example in every day clinical practice to screen for certain diseases or to diagnose a disease, or in clinical research and treatment trials to monitor the activity of diseases and to assess therapeutic response to treatment [5].

Our analysis does not allow us to conclude that any of the mediators tested in this study may be useful as biomarkers of urticaria. However, we do not want to rule out any mediators as a biomarker for CSU. Therefore, we suggest that if biomarkers for urticaria exist, they may only be found by examining larger patients groups from at least two different centres. Furthermore, in addition to the hypothesis-driven approach of assessing individual markers by ELISA, more comprehensive approaches such as proteomics analyses might prove helpful in future investigations.

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