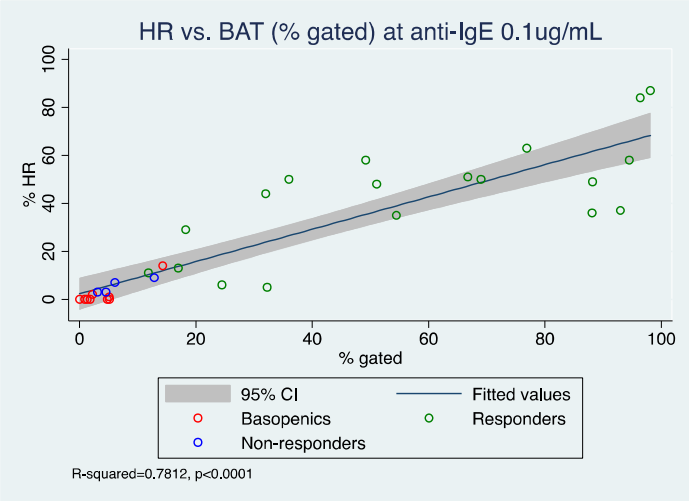
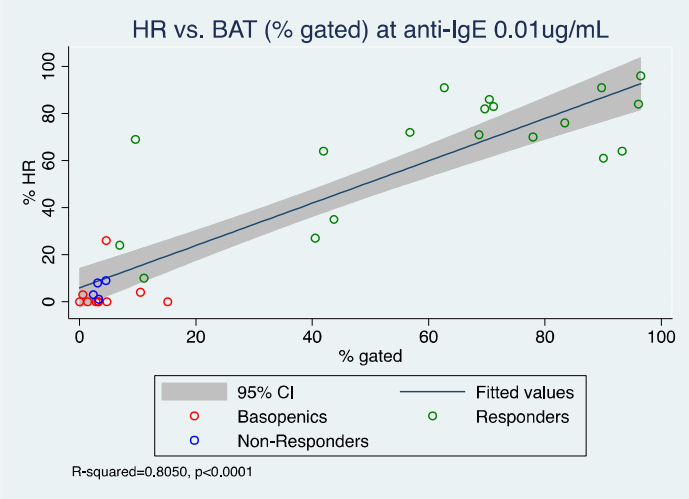
**Figure E1.**

(a)




(b)




(c)

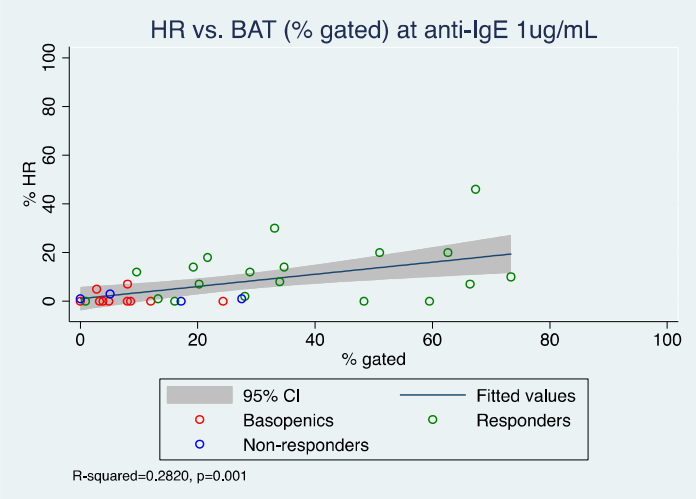


**Table E1.** Demographics and basophil functional characteristics

| Characteristic | Basopenic (n=33) | Responder (n=71) | Non-Responder (n=55) | p-value |
|---|---|---|---|---|
| *Age in years,* Mean (SE) | 41.3 (2.2) | 44.1 (1.7) | 43.7 (1.8) | 0.607 |
| *Gender,* n (%) | | | | |
| Male | 10 (30.3) | 23 (32.4) | 12 (21.8) | 0.419 |
| Female | 23 (69.7) | 48 (67.6) | 43 (78.2) | |
| *Race,* n (%) | | | | |
| White/Caucasian | 25 (75.8) | 53 (74.7) | 46 (83.6) | 0.885 |
| Black/African American | 6 (18.2) | 8 (11.3) | 5 (9.1) | |
| East Asian/Pacific Islander | 0 (0) | 1 (1.4) | 1 (1.8) | |
| American Indian | 0 (0) | 0 (0) | 0 (0) | |
| Hispanic/Latino Origin | 1 (3.0) | 3 (4.2) | 1 (1.8) | |
| South Asian (Indian, etc) | 0 (0) | 2 (2.8) | 1 (1.8) | |
| Other | 0 (0) | 2 (2.8) | 0 (0) | |
| Multi-ethnicity | 1 (3.0) | 2 (2.8) | 1 (1.8) | |
| *Education level*, n (%) | | | | |
| $8^{th}$ grade or less | 0 (0) | 0 (0) | 0 (0) | 0.153 |
| Any high school | 0 (16.4) | 10 (14.3) | 9 (16.4) | |
| Any college graduate | 24 (43.6) | 37 (52.9) | 24 (43.6) | |
| Any post-graduate | 22 (40.0) | 23 (32.9) | 22 (40.0) | |
| Missing | 0 (0) | 1 (1.4) | 0 (0) | |
| *Histamine concentration, ng/1mL blood leukocytes,* Mean (SE) | 1.7 (0.2) | 28.9 (1.8) | 25.7 (2.3) Missing 1 | **<0.001** |
| *Anti-IgE (0.1 ug/mL), % histamine release,* Mean (SE) | --- | 45.0 (2.7) | 2.2 (0.4) | **<0.001** |
| *fMLP ($10^{-6}$ M), % histamine release,* Mean (SE) | **---** | 39.6 (2.6) Missing 1 | 29.5 (2.6) | **0.008** |

**Table E2.** Multivariable regression models adjusting for potential confounders

| Characteristic | Unadjusted Mean | Unadjusted p-value | Adjusted Mean* | Adjusted p-value* |
|---|---|---|---|---|
| *Skindex: emotional component* | | | | |
| Basopenic | (Ref.) | | (Ref.) | |
| Non-responder | -5.6 | **0.014** | -4.5 | **0.046** |
| Responder | -5.5 | **0.012** | -4.4 | 0.050 |
| *Average score for number/size of current hives* | | | | |
| Basopenic | (Ref.) | | (Ref.) | |
| Non-responder | -0.87 | **<0.001** | -0.79 | **0.001** |
| Responder | -0.77 | **0.001** | -0.74 | **0.002** |
| *Current itch* | | | | |
| Basopenic | (Ref.) | | (Ref.) | |
| Non-responder | -2.0 | **<0.001** | -1.8 | **0.002** |
| Responder | -1.1 | **0.041** | -1.0 | 0.072 |
| *Itch during flare* | | | | |
| Basopenic | (Ref.) | | (Ref.) | |
| Non-responder | -0.37 | 0.404 | -0.50 | 0.268 |
| Responder | -1.02 | **0.017** | -1.02 | **0.020** |

| Characteristic | Unadjusted OR | 95% CI | p-value | Adjusted OR* | 95% CI | p-value |
|---|---|---|---|---|---|---|
| *Disease duration < 2 years* | | | | | | |
| Responder | (Ref.) | | | (Ref.) | | |
| Non-responder | 2.8 | **(1.3, 6.1)** | **0.007** | 3.3 | **(1.5, 7.5)** | **0.004** |
| Basopenic | 3.0 | **(1.2, 7.2)** | **0.014** | 3.7 | **(1.4, 9.6)** | **0.007** |
| *One or more steroid tapers in the last year* | | | | | | |
| Responder | (Ref.) | | | (Ref.) | | |
| Non-responder | 2.2 | **(1.04, 4.5)** | **0.038** | 2.2 | **(1.02, 4.9)** | **0.045** |
| Basopenic | 3.5 | **(1.4, 8.8)** | **0.008** | 3.7 | **(1.4, 10.0)** | **0.011** |

*Adjusted for age, gender, race, and education

**Table E3.** Survey Characteristics[1], by functional basophil type

| Survey Characteristic, n(%) | Basopenic (n=33) | Responder (n=71) | Non-Responder (n=55) | p-value[2, 3] |
|---|---|---|---|---|
| *Absent Days in the Past Year,* n (%) | | | | |
| None | 14 (42.4) | 37 (53.6) | 23 (43.4) | 0.43 |
| 1 or more | 19 (57.6) | 32 (46.4) | 30 (56.6) | |
| *Absent Days in Lifetime,* n (%) | | | | |
| None | 9 (27.3) | 19 (28.8) | 17 (32.7) | 0.87 |
| 1 or more | 24 (72.73) | 47 (71.2) | 35 (67.3) | |
| *Hospital/ED Visits in the Past Year,* n (%) | | | | |
| None | 20 (60.6) | 50 (70.4) | 34 (61.8) | 0.47 |
| 1 or more | 13 (39.4) | 21(29.6) | 21 (38.2) | |
| *Hospital/ED Visits in Lifetime,* n (%) | | | | |
| None | 14 (42.4) | 29 (42.0) | 24 (43.6) | 1.0 |
| 1 or more | 19 (57.6) | 40 (58.0) | 31 (56.4) | |
| *Medications ever taken for urticaria,* n (%) | 33 (100) | 69 (97.2) | 55 (100.0) | 0.69 |
| Antihistamine | 28 (84.9) | 53 (74.7) | 40 (72.7) | 0.42 |
| Steroids | 16 (48.5) | 27 (38.0) | 31 (56.4) | 0.12 |
| Leukotrienes | 3 (9.1) | 4 (5.6) | 4 (7.3) | 0.79 |
| Dapsone | 0 (0) | 1 (1.4) | 3 (5.5) | 0.27 |
| Colchicine | 3 (9.1) | 16 (22.5) | 8 (14.6) | 0.22 |
| Sulfasalazine | 19 (57.6) | 28 (39.4) | 30 (54.6) | 0.12 |
| Antidepressants | 8 (24.2) | 8 (11.3) | 12 (21.8) | 0.15 |
| Thyroid medications | 18 (62.1) | 35 (60.3) | 31 (70.5) | 0.58 |
| Stomach acid medications | | | | |
| *Current medications for urticaria,* n (%) | | | | |
| Antihistamine | 26 (89.7) | 50 (86.2) | 35 (79.6) | 0.47 |
| Steroids | 5 (17.2) | 10 (17.2) | 3 (6.8) | 0.25 |
| Leukotrienes | 7 (24.1) | 7 (12.1) | 14 (31.8) | 0.044 |
| Dapsone | 1 (3.5) | 2 (3.5) | 0 (0) | 0.45 |
| Colchicine | 0 (0) | 0 (0) | 0 (0) | --- |
| Sulfasalazine | 2 (6.9) | 7 (12.1) | 5 (11.4) | 0.87 |
| Antidepressants | 8 (27.6) | 9 (15.5) | 11 (25.0) | 0.35 |
| Thyroid medications | 1 (3.5) | 5 (8.6) | 9 (20.5) | 0.078 |
| Stomach acid medications | 6 (20.7) | 14 (24.1) | 16 (36.4) | 0.26 |
| *Family History*, n (%) | | | | |
| Yes | 5 (15.2) | 11 (15.7) | 8 (14.8) | 1.0 |
| *Other allergic diseases,* n (%) | | | | |
| Yes | 4 (20.0) | 18 (43.9) | 9 (29.0) | 0.15 |
| *Systemic symptoms during flare*, | | | | |
| Mean (SD) | 1.82 (0.27) | 1.77 (0.22) | 1.46 (0.21) | 0.52 |
| *# of anaphylaxis symptoms,* mean (SE) | 0.82 (0.13) | 0.77 (0.13) | 0.59 (0.10) | 0.44 |
| *Endorses GI symptoms*, n (%) | 5 (15.2) | 14 (19.7) | 7 (13.0) | 0.60 |
| *Endorses wheezing*, n (%) | 10 (30.3) | 13 (18.3) | 7 (13.0) | 0.14 |
| *Endorses palpitations*, n (%) | 1 (3.0) | 8 (11.3) | 2 (3.7) | 0.25 |
| *Endorses flushing*, n (%) | 11 (33.3) | 20 (28.2) | 16 (30.0) | 0.88 |

[1] Assessed for patients with available data (missing not shown).
[2] Overall p-value comparing the three groups is the first value displayed.
[3] Bolded data indicate values used for sub-comparisons between pairs of groups.
* indicates p-value for basopenics vs. non-responders in sub-comparisons.
** indicates p-value for basopenics vs. responders in sub-comparisons.
*** indicates p-value for responders vs. non-responders in sub-comparisons.

## Supplemental text

### *Methods*

Patient recruitment: Exclusion criteria included use of systemic corticosteroids, cyclosporine, or sulfasalazine in the last month or diagnosis of concomitant urticarial vasculitis, atopic dermatitis, or physical urticaria.

Basophil histamine release (HR) assay: Density gradient sedimentation (single Percoll density centrifugation) was used to isolate blood basophils of patients from venous blood samples.[E1, E 2] Polyclonal goat anti-human IgE (DACI Lab, Baltimore, MD, 0.001-1.0 μg/mL) was used in duplicate to stimulate basophils for histamine release in calcium-containing buffers and measured using automated fluorometry. In addition, basophils were stimulated using N-formyl-met-leu-phe (fMLP) ($10^{-6}$M) as a positive control for basophil degranulation as it is an independent pathway typically preserved in patients with CSU.[E3, E2] Supernatants were collected and analyzed via automated fluorimetry. Results are presented as the percentages of total histamine content of total cell lysates of leukocyte aliquots after spontaneous histamine release was subtracted. Total histamine content was defined as total histamine content released from lysed leukocytes derived from 1mL of whole blood minus the basal level of histamine released spontaneously by basophils without stimulus (i.e. in buffer). Per previously optimized protocol, histamine release levels to 0.1ug/mL concentration of IgE were used to classify basophil functional phenotypes. Patients with histamine release ≥10% of total histamine content were categorized as “responders”, while those with response <10% were categorized as “non-responders”.[E2] Patients with histamine concentrations <5ng/mL blood leukocytes were classified as “basopenics”. Evidence of strong correlation between blood histamine content and

independent verification of basophil numbers by flow cytometry and enumeration has been shown. [E4, E5]

Basophil activation test (BAT): At the time of blood sampling for basophil histamine release, blood samples were also collected in parallel into heparinized tubes (BD vacutainer) for flow studies. Heparinized blood was immediately incubated with either buffer alone or identical doses of anti-IgE antibody or FMLP for 30 minutes at 37°C. Samples were then washed and labeled with CD123 APC (Affymetrix) and either CCR3 PE (Affymetrix) or CD63 PE (Beckman Coulter). Cells were subsequently lysed and fixed (Beckman Coulter) and then analyzed on a BD FACS Calibur flow cytometer. Basophils were gated using CCR3+, CD123+ cell subset and results expressed as both net MFI and % positive relative to buffer. [E6] Exogenous IL-3 was not added to the BAT assay buffers, in contrast to the Rauber et al study, in order to maintain consistent conditions between the HR results and BAT results. [E3]

Written questionnaire: A three-part survey was administered to patients on the same visit as their venipuncture. [E1] Part one included demographic variables, CSU disease duration, family history of CSU, medication usage, as well as CSU-related work/school absenteeism and hospital/ED visits. Part two was the urticaria severity score (USS), an instrument used in previous studies of CSU to quantify wheal size and number and itching in the present time and during flares. [E7, E8] Systemic symptoms during flares were queried, with four classified as being related to anaphylaxis: gastrointestinal, wheezing, palpitations, and flushing. Part three was the SkinDex-29 questionnaire, a validated dermatology instrument for assessing impact of skin disease on quality-of-life in the past 3 months. [E9]

Statistical analysis: Groups means were compared using ANOVA test while proportions of categorical variables were compared using chi-square and Fisher's exact test. For results

showing significant differences, post-hoc pairwise comparisons were conducted using the Bonferroni method. Multivariable linear/logistic regression were used to adjust for potential confounders. Correlation between percent histamine release (% HR) and the basophil activation test (BAT) were assessed by linear regression. Various threshold values were tested to determine the sensitivity and specificity of BAT in identifying basophil phenotypes, with classification by %HR as the gold standard. All analyses were performed in Stata/IC 15.1 (StataCorp, College Station, TX).

***Results (Table E1 and E3)***

Clinical demographics were similar among groups (**Table E1**). By definition, histamine concentration per 1mL blood leukocytes were different among the 3 groups ($p<0.001$) and anti-IgE histamine release was higher in responders compared to non-responders ($p<0.001$). In contrast to previous studies[E1] with smaller samples, lower fMLP histamine release was reported in non-responders (29.5) compared to responders (39.6) ($p=0.008$).

Mean scores for "Symptom" and "Functional" domains of the Skindex-29 were similar between groups ($p=0.14$, 0.17 respectively) (**Table E3**). Absent days due to urticaria in the past year and lifetime were not different ($p=0.43$ and $p=0.87$, respectively). Frequency of hospital/ED visits for urticaria in the past year and lifetime were also not different ($p=0.47$ and $p=1.0$, respectively). No differences were seen between groups in the proportions of patients with positive personal history of allergic co-morbidities ($p=0.15$) or in proportions of patients with positive family history, with roughly 15% positive in all groups ($p=1.0$). No differences were seen among groups in any of the other (i.e. non-steroidal) medications taken for urticaria, either currently or lifetime. Lifetime requirements for one or more steroid tapers was similar ($p=0.45$). Mean score of number and size of hives during flares as well as mean number of wheal locations

during flares were not significantly different among groups (p=0.32, p=0.44 respectively). Duration of urticarial wheals during flares was similar (p=0.32). Mean number of systemic symptoms during a flare was not significantly different among the other groups (p=0.52). Similarly, the mean number of anaphylaxis type symptoms (GI, wheezing, palpitations, flushing) was not significantly different from other groups (p=0.52). Proportions of each of the four symptoms of anaphylaxis were also not different between groups (p>0.05). In all groups, flushing was the most common anaphylaxis-associated symptom while palpitations was the least common.

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**Figure E1.** *Percent histamine release (HR) versus basophil activation test (BAT), at various concentrations of anti-IgE stimuli.* (a) Gold standard for HR: anti-IgE 0.1ug/mL (b) Lower concentration: anti-IgE 0.01ug/mL (c) Higher concentration: anti-IgE 1ug/mL