

A very low number of circulating basophils is predictive of a poor response to omalizumab in chronic spontaneous urticaria

To the Editor:

Chronic spontaneous urticaria (CSU) is characterized by the spontaneous appearance of wheals, angioedema or both for more than 6 weeks.¹ CSU signs and symptoms, albeit not life-threatening, markedly affect patients' quality of life.¹ Mast cell activation and degranulation with subsequent release of inflammatory mediators play a key role in CSU symptom development; however, several points of evidence suggest that basophils also contribute to the disease.^{1,2} Omalizumab, a recombinant humanized monoclonal anti-IgE antibody, represents an effective treatment of CSU and is often used in patients unresponsive to high doses of H1 antihistamines.¹ However, the response to treatment is variable, as some CSU patients respond to treatment within days of their first omalizumab injection, others respond within weeks, and a subgroup of patients do not respond to omalizumab at all.^{1,2} The aim of this study was to evaluate the predictive value of basophil-associated markers, measured before starting omalizumab treatment, for the response to omalizumab in patients with CSU.

In this prospective study, we enrolled 43 patients with active CSU referred to the University Clinic of Respiratory and Allergic Diseases Golnik (Slovenia), whose symptoms were not controlled with high doses of H1 antihistamines and were consequently treated with omalizumab. Their median age was 48 (range, 22–81) years, 70% were women, and the median urticaria activity score 7 (UAS7) before the start of omalizumab treatment was 28 (range, 12–42). The demographic, clinical and laboratory features of CSU patients are summarized in Table 1. The study was approved by the Slovenian National Medical Ethics Committee (KME78/09/14), and all patients gave their informed consent. With the exception of two patients who received 150 mg, all patients received 3 subcutaneous doses of 300 mg omalizumab at 4-week intervals. Response to treatment was defined as a sustained improvement of CSU symptoms (UAS7 ≤ 6) within 2 weeks (early complete responders) or 12 weeks (late complete responders), while no response (NR) was defined as no improvement or only minor improvement of CSU symptoms within 12 weeks. Before the start of omalizumab treatment, total serum IgE; absolute cell count of basophils, lymphocytes, monocytes and granulocytes; CD63 basophil activation and CD63 activation of donor basophils after stimulation with sera of CSU patients; Fc ϵ RI and IgE quantification on the basophil surface; and whole-blood gene expression of basophil-related markers (*FCER1A*, *CPA3* and

HDC) were determined as previously described.^{3,4} The basophil gene mean was calculated as the mean of standardized gene expression variables of *FCER1A*, *CPA3* and *HDC*. For detailed overview of study methodology, refer to Appendix S1.

The rate of clinical response to omalizumab treatment was 84% (36/43). Among CSU responders (CSU-R), 23 (53%) patients were classified as early complete responders and 13 (30%) as late complete responders. Seven (16%) patients were nonresponders (CSU-NR) (Figure S1). No significant differences in age, gender distribution, body mass index (BMI), disease duration or baseline UAS7 were found between CSU-R and CSU-NR (Table 1). However, CSU-NR had significantly lower absolute numbers of circulating basophils and basophil CD63 activation, higher CD63 activation of donor basophils after incubation with sera of CSU patients, lower Fc ϵ RI and IgE densities on basophils, lower whole-blood relative gene expression of *FCER1A* and *HDC*, and a lower basophil gene mean (Table 1). No significant differences were observed in absolute numbers of blood lymphocytes, monocytes, and granulocytes, total serum IgE levels, and whole-blood gene expression of *CPA3*.

As indicated by the estimated area under the ROC curve, the absolute blood basophil count (AUC = 0.99, 95% CI 0.96–1.00), followed by the Fc ϵ RI density per basophil (AUC = 0.95, 95% CI 0.88–1.00), *FCER1A* gene expression (AUC = 0.95, 95% CI 0.89–1.00) and the basophil gene mean (AUC = 0.95, 95% CI 0.89–1.00), showed the highest accuracy in discriminating between CSU-R and CSU-NR (Figure 1A). With a cut-off of >1.4 cells/ μ L (90% CI 0.76–2.45 cells/ μ L), the estimated sensitivity and specificity of the basophil count were 100% and 92%, respectively, compared with 86% and 97% for Fc ϵ RI density per basophil (cut-off, >8691), 100% and 94% for *FCER1A* gene expression (cut-off, >0.05), and 100% and 91% for the basophil gene mean (cut-off, >–0.86). For detailed information on discrimination performance, refer to Table S1.

The number of circulating basophils was the most significant predictor of CSU-NR. Figure 1B describes ORs (with 95% CI) for predicting CSU-NR according to different thresholds of circulating basophils, demonstrating almost complete separation between responders and nonresponders. The optimal cut-off was 1.7 basophils/ μ L with an OR of 144 (95% CI 13–20221). It is also important to note that the 95% CI crosses the neutrality line (OR = 1) at approximately 10 basophils/ μ L (Figure 1B). For detailed information on penalized logistic regression and the stepwise selection model, refer

TABLE 1 Demographic, clinical and laboratory characteristics of CSU patients

Characteristics	CSU	CSU-R	CSU-NR	P value ^a (CSU-R vs CSU-NR)
Patients: N	43	36	7	
Age in years: Median (range)	48 (22-81)	44 (22-81)	52 (41-59)	.589
Gender: N (%)				
Male	13 (30.2)	12 (33.3)	1 (14.3)	.412
Female	30 (69.8)	24 (66.7)	6 (85.7)	
BMI (kg/m ²): Median (range)	26.9 (19.5-45.0)	26.9 (19.5-45.0)	27.9 (22.1-33.9)	.693
UAS7: Median (range)	28 (12-42)	27 (12-42)	42 (26-42)	.041
Disease duration in years: Median (range)	3 (0-25)	3.5 (0-25)	2 (1-25)	.693
Total serum IgE (kU/L): Median (range)	60.5 (<1-1186)	87 (<1-1186)	20 (2-146)	.005
Absolute basophil count (cells/ μ L): Median (range)	6.8 (0.1-31.4)	8.6 (0.3-31.4)	0.2 (0.0-1.4)	<.0001
Absolute lymphocyte count (cells/ μ L): Median (range)	1443 (27-2835)	1538 (27-2835)	1050 (655-1906)	.049
Absolute monocyte count (cells/ μ L): Median (range)	192 (14-504)	191 (14-504)	196 (47-409)	.815
Absolute granulocyte count (cells/ μ L): Median (range)	3106 (152-6920)	2934 (152-6151)	3571 (1675-6920)	.392
CD63 basophil activation (%): Median (range)	2.4 (0-17.4)	2.5 (0.0-17.4)	0.0 (0.0-7.7)	.001
CD63 activation of donor basophils (%) after incubation with sera of CSU patients: Median (range)	1.7 (0.0-94.0)	1.4 (0.0-60.2)	20.5 (10.4-94.0)	.0003
N, (%) of >10% activated basophils	13 (30.2)	6 (16.7)	7 (100)	<.0001
Fc ϵ RI density per basophil: Median (range)	67 080 (1586-166 548)	80 524 (4082-166 548)	5110 (1586-33 295)	<.0001
IgE density per basophil: Median (range)	64 220 (1213-218 377)	85 821 (1213-218 377)	4102 (1652-29 191)	<.0001
Relative mRNA expression in whole blood (fold change): Median (range)				
FCER1A	0.24 (0.00-2.28)	0.27 (0.02-2.28)	0.03 (0.00-0.05)	<.0001
CPA3	0.17 (0.01-3.19)	0.22 (0.02-3.19)	0.04 (0.01-0.32)	.016
HDC	0.11 (0.00-1.79)	0.16 (0.01-1.79)	0.01 (0.00-0.03)	.0007
Basophil gene mean ^b	-0.05 (-1.66-1.65)	0.32 (-1.11-1.65)	-1.06 (-1.66-0.86)	<.0001

Note: Laboratory measurements and/or sampling were performed at baseline before the start of omalizumab treatment.

No significant differences in demographic, clinical and laboratory characteristics were observed between early and late complete responders (data not shown).

Abbreviations: BMI, body mass index; CPA3, carboxypeptidase A3; CSU, chronic spontaneous urticaria; CSU-NR, nonresponders to omalizumab treatment; CSU-R, responders to omalizumab treatment; FCER1A, the high affinity IgE receptor; HDC, L-histidine decarboxylase; N, number of patients; UAS7, Urticaria Activity Score 7.

^aP values were Bonferroni-corrected, and a P value < .003 was accepted as significant. Statistically significant P values are in bold face.

^bThe basophil gene mean was calculated as the mean of standardized gene expression variables of FCER1A, CPA3 and HDC.

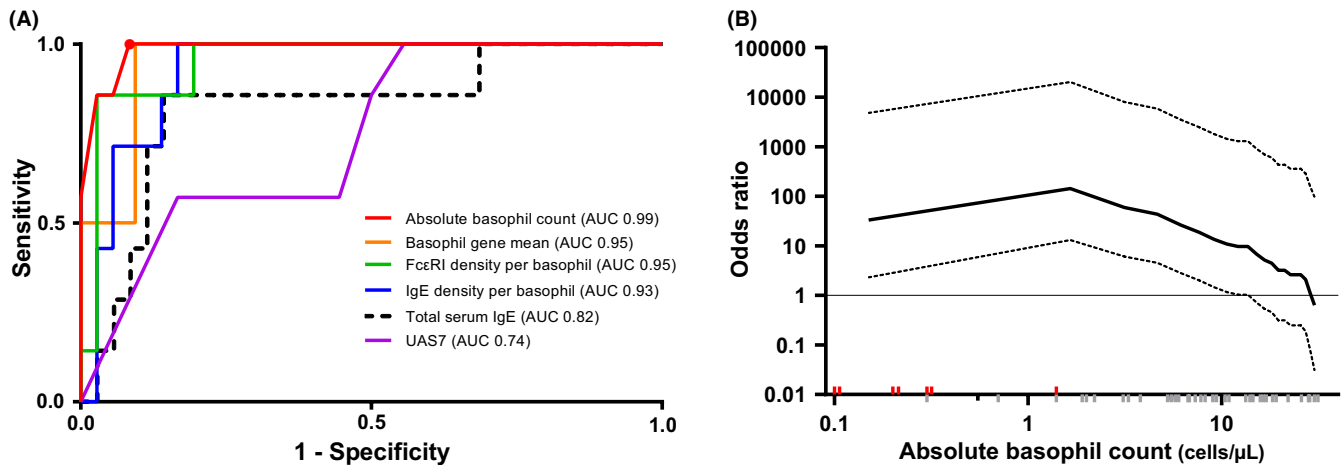


FIGURE 1 (A) ROC curves comparing the discriminating performance of selected analysed parameters and the (B) OR (full line) and 95% CI (dashed lines) of a binominal predictor using number of circulating basophils as cut-offs for predicting nonresponders among 43 CSU patients treated with omalizumab, of whom 36 were responders (marked grey) and seven were nonresponders (marked red)

to Table S2. Additionally, a very low number of circulating basophils significantly correlated with very low densities of FcεRI and IgEs on basophils (Figure S2).

Previous studies have shown that serum total IgE,^{5,6} FcεRI density per basophil⁷ and activation of donor basophils after incubation with sera of CSU patients⁸ have been proposed as predictors of the response to omalizumab therapy in CSU. Our results confirmed the possible importance of tIgE, basophil FcεRI and IgE density, and the autoimmune basophil response. Basopenia reported in patients with CSU is postulated to be the result of migration of basophils from the blood into the skin.^{2,9} Rauber et al⁴ suggested that in CSU patients distinct basophil phenotypes might be associated with different responses to therapies, and Saini et al⁹ demonstrated that clinical improvement observed with omalizumab treatment may be related to an increase in the number of circulating basophils. The proposed mechanism is that omalizumab changes the behaviour of basophils by lowering the densities of FcεRI and IgEs on basophils, possibly affecting their migration.^{2,9} Therefore, a subgroup of CSU patients, characterized by very low number of circulating basophils, autoimmune basophil response and autoreactive serum are less likely to respond to omalizumab treatment. However, the indication that specific circulating basophil characteristics may be important for the clinical response does not necessarily indicate a causal relationship between basophils and disease pathophysiology.

In conclusion, the most important finding of our study is that a very low number of circulating basophils, in addition to the corresponding low whole-blood gene expression of basophil-related markers, especially *FCER1A* and the basophil gene mean, showed the highest accuracy in discriminating between CSU-R and CSU-NR, and in predicting the response to omalizumab therapy. Therefore, we speculate that the baseline absolute basophil count represents a promising and feasible laboratory approach for

predicting the clinical response to omalizumab. However, a substantially broader assessment is required to validate and replicate this finding.

KEYWORDS

basophils, chronic spontaneous urticaria, FcεRI, gene expression, omalizumab

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to the contents of this work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

APPENDIX S1

SUPPORTING INFORMATION

Supplementary materials and methods

Absolute peripheral blood cell counts and basal CD63 basophil activation

For the absolute basophil count (CD123⁺/HLA-DR⁻ cells; BD Biosciences, San Jose, CA, USA), we added AccuCount Fluorescent microbeads (Spherotec, Inc., Libertyville, IL, USA) to fixed samples before flow cytometric analysis. Lymphocytes, monocytes, and granulocytes were gated according to lysed whole-blood forward-scatter/side-scatter characteristics. Absolute numbers of basophils, lymphocytes, monocytes, and granulocytes per microliter of whole blood were calculated by using the following equation: (number of cells/number of events for each microbead region) × (number of microbeds used in test/volume of the whole blood sample)^{1,2}.

A basophil activation assay was performed on heparinized whole blood incubated with basophil stimulation buffer with IL-3 (Buhlmann, Switzerland) at 37°C for 15 minutes. Degranulation was stopped by chilling on ice, after which anti-CD63, anti-CD123, and anti-HLA-DR mAbs (BD Biosciences) were added and incubated for 20 minutes. Finally, whole blood probes were lysed, washed, fixed, and analysed within 2 hours on a flow cytometer¹. The basophils were identified as low side scatter, CD123-positive, and HLA-DR-negative cells, and the quantitative percentage of degranulated basophils was determined as a percentage of the CD63-positive cells.

Activation of donor basophils after incubation with sera of CSU patients

To detect autoreactive serum components in patients with CSU, 50 µl of heparinized whole blood from healthy donors was incubated with 50 µl of CSU patient sera and basophil stimulation buffer with IL-3 at 37°C for 15 minutes. Degranulation was stopped by chilling on ice, after which anti-CD63, anti-CD123, and anti-HLA-DR mAbs (BD Biosciences) were added and incubated for 20 minutes. Finally, whole blood probes were lysed, washed, fixed, and analysed within 2 hours on a flow cytometer^{1,3}. The basal CD63 activation of donor basophils was 1.5 – 2.5%.

FcεRI and IgE quantification on the basophil surface

FcεRI and IgE quantification on the basophil surface was performed as previously described in detail^{1,4}. Briefly, the density of FcεRI receptors or IgEs on the basophil surface (PE-conjugated CD123-positive and PerCP-conjugated HLA-DR-negative cells; BD Biosciences) was analysed using a FITC-conjugated antibody to FcεRI (Thermo Fisher Scientific) or anti-IgE (Miltenyi Biotec, Bergisch Gladbach, Germany) and calculated with comparison to the standard curve of calibration beads (Agilent, Santa Clara, CA, USA). With assigned molecules of equivalent fluorochrome (MEF) values for the fluorescent bead populations, arbitrary units of FITC mean fluorescence intensity (MFI) were transformed into absolute units.

Total IgE measurements

The measurements of total IgE from sera samples of patients were determined with an Immulite system (Siemens, Erlangen, Germany).

Gene expression

Total RNA was isolated from whole-blood samples by using the PAXgene Blood miRNA Kit (PreAnalytiX) according to the manufacturer's instructions and as previously described². Following reverse transcription using a high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), cDNA was quantified by using real-time PCR (ABI PRISM 7500 Real-Time PCR System; Applied Biosystems, Foster City, Calif) under standard conditions with TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific). TaqMan assays FCER1A (Hs00175232_m1), CPA3 (Hs00157019_m1), and HDC (Hs00157914_m1) were utilized to quantify *FCER1A*, *CPA3* and *HDC* mRNA expression levels, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 4333764) was used as an endogenous control (Applied Biosystems). All measurements were performed in triplicate for each sample, and relative expression was analysed using the $\Delta\Delta C_t$ method.

The basophil gene mean was calculated as the mean of standardized gene expression variables of *FCER1A*, *CPA3* and *HDC*. Gene expression of *FCER1A*, *CPA3* and *HDC* were first centred and scaled, and then a mean value of these standardized variables was calculated to generate a single value representing the mean expression of basophil related genes, called the basophil gene mean. This methodology was previously described to calculate the »gene mean«⁵.

The high affinity IgE receptor (FcεRI) consists of 3 subunits: α , β , and γ . FcεRI is expressed on mast cells and basophils as tetramers ($\alpha\beta\gamma_2$) and on antigen-presenting cells, although at substantially lower levels, as trimers ($\alpha\gamma_2$). *FCER1A* encodes the α subunit. Carboxypeptidase A3 (encoded by *CPA3*) is expressed in mast cells and basophils and can be expressed in populations of T-cell progenitors and thymic T cells and in some hematopoietic progenitor cells. L-histidine decarboxylase (encoded by *HDC*) catalyzes the formation of histamine from L-histidine, and in hematopoietic cell lineages the gene is expressed only in mast cells and basophils.

Statistical analysis

Data distribution was evaluated using the D'Agostino–Pearson test. Parametric statistics (unpaired t-tests) were used on normally distributed data, whereas non-parametric statistics (the Mann–Whitney) were used if the distribution deviated from normal. Gender distribution was compared using Fisher's exact test based on a contingency table. The performances of biomarkers to discriminate between CSU-R and CSU-NR were compared using receiver operating characteristic (ROC) curve analysis, and the optimal cut-off was determined by using Youden's index. Spearman's rank correlation test was used to analyse the correlation between analysed parameters. Because of the low prevalence of the outcome variable (7 nonresponders in the whole cohort), we used, for prediction purposes, a penalized logistic regression model⁶. Linear combination of predictors for AUC optimisation was performed based on AUC maximization strategy with cross validation on log-transformed data⁷. GraphPad Prism 8.0.1 software (San Diego, CA, USA) and R^{8,9,10} and its affiliated packages were used for statistical analysis. *P values* were conservatively Bonferroni-corrected for the total number of parameters tested (18), and a *P value* < .003 was accepted as significant.

Supplementary results

Predictors of CSU-NR

We first performed univariate regression (Table S2) of all the predictors to see how they separately correlate to the outcome variable. To find the best model to describe the sampled data, we used forward stepwise regression with a blank model as a starting model and then added one predictor at a time in a stepwise manner; the addition of the predictor was based on the penalized likelihood ratio test. The best model was the one that used only the number of

basophils as the predictor in the model – adding other predictors did not significantly improve the prediction power of the model.

We have also analysed the discrimination performance of combination of different parameters/tests and performed an estimation of AUC based on the linear combination of log transformed data (Table S3), demonstrating that linear combinations of different predictors did not have superior discrimination capabilities than single predictors, probably because the single predictors by itself were powerful discriminators. Even though, with the combination of Absolute basophil count/IgE density per basophil/FcεRI density per basophil/FCER1A/Total serum IgE/UAS7 we have and AUC of 0.999.

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TABLE S1 Discrimination performance of different parameters/tests for the response to omalizumab therapy in CSU patients

	Optimal cut-off*	AUC ROC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)
UAS7	23	0.74 (0.56-0.93)	100 (59-100)	44 (28-62)	1.80 (1.3-2.4)	0.00
Total serum IgE	35.7	0.82 (0.64-1.00)	86 (42-100)	86 (70-95)	6.00 (2.5-14.3)	0.17 (0.03-1.00)
Absolute basophil count	1.4	0.99 (0.96-1.00)	100 (59-100)	92 (78-98)	12.00 (4.1-35.5)	0.00
Absolute lymphocyte count	1443	0.74 (0.53-0.94)	86 (42-100)	56 (38-72)	1.93 (1.2-3.1)	0.26 (0.04-1.6)
Absolute monocyte count	191	0.53 (0.28-0.78)	71 (29-96)	53 (36-70)	1.51 (0.8-2.7)	0.54 (0.2-1.8)
Absolute granulocyte count	5022	0.61 (0.29-0.92)	43 (10-82)	97 (86-100)	15.43 (1.9-127.7)	0.59 (0.3-1.1)
CD63 basophil activation	0	0.80 (0.54-1.00)	86 (42-100)	86 (71-95)	6.17 (2.6-14.7)	0.17 (0.03-1.0)
CD63 activation of donor basophils after incubation with sera of CSU patients	10.2	0.90 (0.81-1.00)	100 (59-100)	86 (71-94)	7.20 (3.2-16.2)	0.00
FcεRI density per basophil	8691	0.95 (0.88-1.00)	86 (42-100)	97 (86-100)	30.86 (4.4-218.2)	0.15 (0.02-0.9)
IgE density per basophil	29191	0.93 (0.85-1.00)	100 (59-100)	83 (67-94)	6.00 (2.9-12.5)	0.00
Relative mRNA expression:						
<i>FCER1A</i>	0.05	0.95 (0.89-1.00)	100 (54-100)	94 (79-99)	16.00 (4.2-61.2)	0.00
<i>CPA3</i>	0.04	0.81 (0.61-1.00)	67 (22-96)	88 (71-97)	5.33 (1.4-20.4)	0.38 (0.1-1.2)
<i>HDC</i>	0.03	0.91 (0.81-1.00)	100 (54-100)	81 (64-93)	5.33 (2.6-11.0)	0.00
Basophil gene mean	-0.86	0.95 (0.89-1.00)	100 (54-100)	91 (75-98)	10.67 (3.6-31.3)	0.00

AUC, Area under the curve; PLR: Positive likelihood ratio; ROC, Receiver operating characteristic; NLR, Negative likelihood ratio; UAS7, Urticaria Activity Score 7.

* Optimal cut-offs were determined by using the Youden index.

TABLE S2 Univariate regression analysis of all the predictors for the response to omalizumab therapy in CSU patients

	OR (95% CI)	<i>P</i> value [‡]
Age in years	0.98607 (0.92601-1.04783)	.648
BMI	0.97849 (0.8606-1.13901)	.753
UAS7	0.91373 (0.81605-0.99908)	.048
Total serum IgE	1.01611 (0.99927-1.04494)	.100
Absolute basophil count	5.60394 (1.5085-67.14218)	< .001
Absolute lymphocyte count	1.00128 (0.99985-1.0031)	.081
Absolute monocyte count	0.9986 (0.99107-1.00686)	.721
Absolute granulocyte count	0.9995 (0.99888-1.00007)	.086
CD63 basophil activation	1.54865 (0.9094-3.02958)	.172
CD63 activation of donor basophils after incubation with sera of CSU patients	0.96117 (0.92337-0.99192)	0.013
FcεRI density per basophil	1.00008 (1.00003-1.00021)	< .001
IgE density per basophil	1.00006 (1.00002-1.00014)	< .001
Relative mRNA expression:		
<i>FCER1A</i>	1.78375×10 ¹⁵ (2653-4.383789×10 ³⁹)	.001
<i>CPA3</i>	18.68702 (0.69296-143689.1)	.136
<i>HDC</i>	1.657707×10 ¹³ (7.07946-2.457316×10 ⁶⁹)	.011
Basophil gene mean [†]	63.22852 (3.45861-90626.05)	< .001

CSU, Chronic spontaneous urticaria; BMI, Body Mass Index; UAS7, Urticaria Activity Score 7; FCER1A, The high affinity IgE receptor; CPA3, Carboxypeptidase A3; HDC, L-histidine decarboxylase.

[†]The basophil gene mean was calculated as the mean of standardized gene expression variables of *FCER1A*, *CPA3* and *HDC*.

[‡]*P* values were Bonferroni-corrected, and a *P* value < .003 was accepted as significant. Statistically significant *P* values are in bold face.

TABLE S3 Discrimination performance of combinations of different parameters/tests for the response to omalizumab therapy in CSU patients

	AUC optimistic	AUC pessimistic	Linear combination
Absolute basophil count/ <i>FCER1A</i>	0.992	0.99	-0.852/-0.523
Absolute basophil count/IgE density per basophil/ <i>FCER1A</i>	0.993	0.992	-0.923/-0.225/-0.311
Absolute basophil count/IgE density per basophil/Total serum IgE	0.996	0.992	-0.644/-0.646/0.41
Absolute basophil count/ <i>FCER1A</i> /UAS7	0.992	0.992	-0.878/-0.452/0.156
Absolute basophil count/IgE density per basophil/FcεRI density per basophil/tIgE	0.995	0.993	-0.72/-0.325/-0.435/0.434
Absolute basophil count/IgE density per basophil/ <i>FCER1A</i> /Total serum IgE	0.998	0.997	-0.626/-0.593/-0.361/0.356
Absolute basophil count/IgE density per basophil/ <i>FCER1A</i> /UAS7	0.993	0.991	-0.926/-0.171/-0.331/0.066
Absolute basophil count/IgE density per basophil/Total serum IgE/UAS7	0.996	0.993	-0.609/-0.623/0.457/0.18
Absolute basophil count/FcεRI density per basophil/ <i>FCER1A</i> /Total serum IgE	0.991	0.989	-0.802/-0.422/-0.335/0.257
Absolute basophil count/FcεRI density per basophil/Total serum IgE/UAS7	0.992	0.988	-0.578/-0.614/0.488/0.226
Absolute basophil count/ <i>FCER1A</i> /Total serum IgE/UAS7	0.992	0.991	-0.877/-0.432/0.053/0.205
IgE density per basophil/FcεRI density per basophil/Total serum IgE /UAS7	0.991	0.985	-0.508/-0.576/0.608/0.201
Absolute basophil count/IgE density per basophil/FcεRI density per basophil / <i>FCER1A</i> /Total serum IgE	0.999	0.997	-0.533/-0.589/-0.234/-0.333/0.45
Absolute basophil count/IgE density per basophil/FcεRI density per basophil/Total serum IgE/UAS7	0.998	0.997	-0.406/-0.55/-0.363/0.608/0.178
Absolute basophil count/IgE density per basophil/ <i>FCER1A</i> /Total serum IgE/UAS7	0.999	0.998	-0.541/-0.54/-0.352/0.463/0.278
Absolute basophil count/FcεRI density per basophil / <i>FCER1A</i> /Total serum IgE/UAS7	0.993	0.992	-0.82/-0.33/-0.266/0.312/0.225
IgE density per basophil/FcεRI density per basophil/ <i>FCER1A</i> /Total serum IgE/UAS7	0.995	0.99	-0.464/-0.434/-0.409/0.59/0.285
Absolute basophil count/IgE density per basophil/FcεRI density per basophil/ <i>FCER1A</i> /Total serum IgE/UAS7	0.999	0.999	-0.447/-0.515/-0.211/-0.32/0.561/0.272

AUC, Area under the curve; *FCER1A*, Relative mRNA expression of *FCER1A*; UAS7, Urticaria Activity Score 7.

FIGURE S1 The effect of omalizumab treatment on UAS7. Results are expressed as the median with IQR. Mann-Whitney U test for CSU-R vs CSU-NR; **** $P < .0001$. CSU-R, responders to omalizumab treatment; CSU-NR, nonresponders to omalizumab treatment.

FIGURE S2 Correlation heatmap reporting Spearman correlation coefficients and *P values* for each comparison. The bar on the left side of the map indicates the colour legend of the Spearman correlation coefficients, and the bar on the right side of the map indicates the colour legend of the *P values* calculated for each pair of samples in the matrix. *P values* were Bonferroni-corrected, and a *P value* $< .003$ was accepted as significant. Non-significant *P values* $> .003$ are presented in grey squares.

