Quantity increase and functional affinity/avidity decrease of anti-FcεRI and anti-IgE autoantibodies in chronic spontaneous urticaria

Short Title: Quantity and avidity of anti-FcεRI and anti-IgE autoantibodies in chronic spontaneous urticaria

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Abstract

Background: Patients with autoimmune forms of chronic spontaneous urticaria (aiCSU) exhibit autoantibodies against the high-affinity IgE receptor (FcεRI) and IgE. As the presence of these autoantibodies does not correlate with disease activity, the functional affinity/avidity may be relevant in aiCSU. This exploratory study aimed to characterize the quantity and avidity of autoantibodies against IgE and FcεRI over 6 months.

Methods: The serum of 49 patients with CSU and 30 healthy control subjects was obtained at baseline and 6 months. Serum was analyzed by ELISA, to determine the quantity and avidity of anti-IgE and anti-FcεRI autoantibodies, and by basophil activation test (CU-BAT).

Results: An increase in the quantity of anti-FcεRI and anti-IgE antibodies and a simultaneous decrease in avidity was found in all patients with CSU after 6 months: median anti-IgE increased from 6.7 ng/mL (IQR 5.1-12.5) to 23.8 ng/mL (IQR 12.3-121.5), $p<0.001$, median anti-FcεRI from 52.4 ng/mL (IQR 26.3-111.4) to 129.5 ng/mL (IQR 73.7-253.7), $p<0.001$. Median anti-IgE avidity decreased from 75.8% (IQR 55.3-90.8) to 56.4% (IQR 30.6-76.2), $p=0.019$ and median anti-FcεRI avidity from 75.1% (IQR 49.8-90.0) to 52.2 (IQR 38.2-60.1), $p<0.001$. In contrast, the frequency of activated basophils did not change significantly over time. Surprisingly, autoantibody avidity did not correlate with basophil activation.

Conclusion: Both the quantity and avidity of anti-FcεRI and anti-IgE antibodies change over time, demonstrating that the CU-BAT is more suitable to diagnose aiCSU. In addition, the avidity of anti-FcεRI and anti-IgE antibodies do not correlate with CU-BAT and disease activity, suggesting that further factors independent of anti-FcεRI and anti-IgE autoantibodies contribute to aiCSU.

Impact statement

The quantity and avidity of anti-FcεRI and anti-IgE autoantibodies are dependent on the time of analysis in CSU and are therefore of limited use for the diagnosis of autoimmune forms of CSU.

Keywords

Affinity, avidity, basophil activation test, chronic idiopathic urticaria, chronic spontaneous urticaria, FcεRI receptor, anti-FcεRI, anti-IgE, autoantibodies, autoimmune

Introduction

Chronic spontaneous urticaria (CSU) is a common skin disease characterized by redness, itchy hives, and angioedema lasting at least 6 weeks (1). Although the exact pathogenesis of CSU is not completely understood, autoimmunity has been proposed in a proportion of patients based on the presence of autoreactive serum components and mast cell activation, as demonstrated by the autologous serum skin test (ASST) (2). The basophil activation test (CU-BAT) was recently established as a specific, sensitive, and safe substitute for the ASST (3).

Approximately one-third to one-half of patients with CSU have IgG autoantibodies directed against IgE or the high-affinity IgE receptor, FcεRI (4). A study also identified IgA and IgM autoantibodies against FcεRI in some patients with CSU (5). It is hypothesized that anti-IgE and anti-FcεRI antibodies play a major role in the pathogenesis of some forms of CSU by activating the FcεRI pathway. Therefore, patients with CSU with a positive ASST/BAT and/or anti-IgE and anti-FcεRI antibodies are subclassified as autoimmune CSU type II (aiCSU) (6). Another CSU subtype is driven by a type I IgE-mediated autoallergy mechanism (aaCSU) and should be distinguished from aiCSU (6). Instead of IgG autoantibodies, patients with aaCSU exhibit IgE autoantibodies against thyroperoxidase, double-stranded DNA, interleukin-24, tissue factor, and thyroglobulin (7-10).

Interestingly, autoantibodies against IgE and FcεRI can be found in patients with other autoimmune diseases and even in healthy individuals (11). Furthermore, autoantibody levels in CSU patients do not change significantly over time, despite undulating disease activity (12). This suggests, that these autoantibodies may not be functional. One possibility could be that a second signal or primer, in addition to anti-IgE/anti-FcεRI autoantibodies, is required to trigger basophil/mast cell activation in aiCSU (13). Alternatively, the affinity/avidity of the autoantibodies may be higher in patients with aiCSU due to somatic hypermutation (14). While somatic hypermutation and high-affinity antibodies are beneficial in an immune response to infectious pathogens, high-affinity autoantibodies can exacerbate disease activity in autoimmune diseases (15). The majority of studies focus on the affinity/avidity of antibodies against infectious pathogens (16-18). However, autoantibodies have been better characterized in recent years, especially antiphospholipid antibodies and those against nervous tissue (19; 20). Therefore, determining the quantity and avidity of the anti-IgE and anti-FcεRI autoantibodies in CSU could be of prognostic importance.

This study aimed to characterize autoantibodies against IgE and FcεRI in patients with CSU over 6 months and analyze the correlation between autoantibody avidity and basophil activation.

Methods

Study design and patients

This monocentric, prospective exploratory study investigated the quantity and avidity of autoantibodies against IgE and the FcεRI receptor in CSU patients between January 2018 to December 2019. 49 patients with CSU referred to our allergy unit for an allergologic workup were included in this study. All patients had two study visits with a time interval of 6 months, in which CU-BAT, ELISA, and the urticaria control test (UCT) were performed. All patients included gave informed consent. The study was approved by the local ethics committee (Kantonale Ethikkommission Bern: 187/11) and funded by the Ulrich Müller Gierok Allergy Foundation Bern, Switzerland.

Patients between the ages of 18 and 80 with CSU were included in the study if they had symptoms for at least 6 weeks, with hives present at least three times weekly. Patients with inducible or allergic forms of urticaria or documented prior treatment with systemic immunosuppressive agents (except

short-term prednisolone under 7 days for CSU exacerbations with a maximal dose of 50mg prednisolone) were excluded from this study. For comparison purposes, all baseline study procedures were performed in 30 healthy subjects.

Basophil activation test (CU-BAT)

CU-BAT was performed at baseline and at 6 months as previously described (3;21). Briefly, peripheral mononuclear cells from 2 healthy donors were isolated by density gradient centrifugation, suspended in a test solution (RPMI-1640, 1 mmol/L CaCl2, 1 mmol/L MgCl2, and 1% BSA) and primed with IL-3 (1 ng/mL). From this solution, 100 µL were incubated for 30 minutes at 37ºC with 100 µL of CSU serum. An anti-IgE antibody (100 ng/mL; Beckman Coulter, Marseille, France, Clone E124.2.9), N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) (Sigma-Aldrich, St. Louis, USA), a serum mix of healthy donors (n=5), and the test solution acted as positive and negative controls. The cells were stained with phycoerythrin conjugated anti-CCR3 (BioLegend, San Diego, USA; clone 5E8) and anti-CD63-FITC (BioLegend, San Diego, USA; clone H5C6) for 20 minutes at 4ºC, then washed with CellWASH. Basophils were detected by CCR3 expression using a FACSCanto flow cytometer. CD63 served as an activation marker and was expressed as the percentage of activated basophils (mean of two measurements).

Basophil characteristics of healthy donors were examined in line with our prior publication by Gentinetta et al. [3]. In short, healthy donor basophils were tested for their capability to react to different known CU-BAT serum samples as positive control and a serum mix of healthy donors as negative control with gradually increasing IL-3 concentrations to standardize the results across various basophil donors. Overall, slight variations in basophil reactivity were observed among different donors, but using individual IL-3 concentrations for the different donors these differences were no longer statistically significant and did not correlate with IgE receptor density, as previously noted by Gentinetta. In addition, the expression of FcεRIα on basophils is mainly regulated by levels of free IgE, which may vary in response to factors such as allergen exposure, smoking, or other environmental influences (22).

Quantity and avidity of anti-IgE and anti-FcεRI autoantibodies

IgG antibodies against FcεRIα and IgE were evaluated using an ELISA at baseline and 6 months. The ELISA plate was coated overnight with appropriate concentrations of the alpha subunit of FcεRI (Human FceRI/ FceR1A Protein, Lubio Science, Zurich, Switzerland; concentration of 0.73 µg/ml) or human IgE (human IgE, NBS-C Bioscience, Vienna, Austria; clone SUS-11; 0.5 µg/ml). The plate was blocked with 3% milk PBS for two hours at room temperature after washing (PBS+0.05% Tween20) to reduce non-specific binding. A human anti-FcεRI standard (Creative Biolabs, Shirley, USA; Clone NPB311), humanized anti-IgE (omalizumab, Xolair®, Novartis Pharma AG Basel, Switzerland), and the patient serum were diluted with 1% milk PBS, added to the plate in replicates, and incubated for one hour at 37°C. The samples were then incubated (1h, 37°C, 5% CO2) with the detection antibody, a peroxidase-coupled anti-human IgG antibody (Binding Site, Birmingham, UK; AP004), and developed by adding tetramethylbenzidine (TMB) substrate. The development reaction was stopped with sulfuric acid. The absorbance was measured at 450nm, and the number of antibodies was determined from the standard curve.

For the affinity/avidity analysis, half of the replicates of each patient's serum were incubated with chaotropic reagent ammonium thiocyanate (0.5M), and the other half with 1% milk PBS for 10 minutes, shaking at room temperature at 300rpm. Prior to the assay, different concentrations of ammonium thiocyanate (0-1.5M) were tested, and for subsequent testing, a concentration of 0.5M was used. After an immediate washing step, the samples were incubated (1h, 37°C, 5% CO2) with the detection antibody and developed by adding TMB substrate. The absorbance was measured at 450nm. The avidity of the autoantibody was expressed as the percent residual autoantibody quantity at 0.5M of the chaotropic reagent.

Urticaria Control Test (UCT)

UCT scores were measured at baseline and 6 months. The UCT is a validated, retrospective questionnaire with 4 questions to assess disease activity in patients with chronic urticaria during the previous month (23). Each question can be answered with 5 options, scored with 0 to 4 points. The cumulative minimum scores range from 0 to 16, with 16 indicating complete urticaria control.

Posthoc analysis

A subset of study participants were selected for an additional avidity evaluation by surface plasmon resonance assay (SPR) testing (Biacore, Cytiva, Uppsala, Sweden) to compare with the ELISA assay and to provide additional insights into binding interactions. This subset was chosen based on the strength of binding interactions observed with chaotropic reagents and the availability of samples. Only baseline samples were evaluated.

Surface plasmon resonance measurements

All SPR measurements were performed on a GE Healthcare Biacore X100 device (IL, USA). HBS-EP+ was used as a running buffer at a 10 μL/min flow rate. Recombinant human FcεRIα were immobilized on flow cell 2 (Fc2) of a CM5 sensor chip by standard amine coupling at a target level of 1000RU. A blank immobilization was performed with the reference flow cell 1 (Fc1). Binding responses were displayed as Fc2 signals minus binding responses on the Fc1 reference cell. Recombinant human IgE (Sus11-IgE, NBS-C BioScience) at a concentration 20 nM was captured on the immobilized FcεRIα for 120 seconds. Different dilutions (i.e. 1:2, 1:4, 1:8 1:16 and 1:32) of purified IgG fractions (NAb™ Protein G Spin Kit, Thermo Scientifc) from CSU patient sera were injected for 120 seconds with a dissociation time of 180 seconds between each injection under constant buffer flow on the pre-formed IgE:FcεRIα complexes. At the end of each run the chip surface was regenerated for 14 seconds with 25 mM NaOH and reloaded with Sus11-IgE. To determine binding kinetics, we used the BIAevaluation software. Affinity constants were calculated using a 1:1 Langmuir curve fitting model.

Statistical analysis

Baseline characteristics between patients and control participants were compared using a Chi-squared test or non-parametric Wilcoxon rank-sum test, as appropriate. A Wilcoxon signed-rank test was used to compare the patients with CSU at baseline and 6 months. Associations between CU-BAT and autoantibody avidity were calculated using Spearman's rho. For subgroup analysis, all patients with elevated autoantibody titers at baseline were analyzed independently. Cut-offs for subgroup analysis of antibody quantity were determined as the mean plus twice the standard deviation of the control group. Stata 16 (Stata Corporation, College Station, Texas, USA) was used for all statistical analyses, and P values less than 0.05 were considered statistically significant. Some patients had to start

omalizumab treatment before the 6 months follow-up. All the measurements related to quantity and avidity measurements of anti-IgE antibodies (but not CU-BAT or anti- FcεRI) were ignored if the patient was under omalizumab medication due to its influence: omalizumab was the standard of this assay. Associations between autoantibody avidity by chaotropic agents and surface plasmon resonance assay (SPR) were calculated using Spearman's rho.

Results

Study Patients

A total of 49 patients with CSU and 30 healthy subjects were included in this study (Table I). The proportion of males was 29% and 33% in patients with CSU and healthy subjects, respectively. The median age was 35.0 (IQR 25.0-49.0) in patients with CSU, which was slightly higher compared to healthy controls (26.5 (IQR 23.0-39.5); p=0.05). The median disease duration at baseline was short at 7.0 (IQR 4.0-15.0) months. At baseline, 57% of patients with CSU had angioedema, 94% were on antihistamines, 4% on montelukast, 10% on systemic steroids in the 6 months before (1 patient with 40mg prednisolone 6 month, two patients with 50mg 2 and 4 months and two with 20mg prednisolone 1 month prior to inclusion, no patients under systemic steroids at inclusion), and one patient was on omalizumab. At 6 months, 8 patients were under omalizumab treatment, none of the patients had systemic steroid treatment. The median UCT score at baseline was 6.0 (IQR 3.0-8.0) and was significantly higher after 6 months (12.0; IQR 11.0-14.5; p<0.001), indicating lower CSU activity. CSU had subsided in 5 subjects at the 6-month follow-up.

Baseline comparison of the quantity and avidity of anti-FcεRI and anti-IgE autoantibodies and basophil activation

We first compared the quantity and avidity of anti-FcεRI and anti-IgE autoantibodies in patients with CSU and healthy controls at baseline (Table I). The median quantity of anti-IgE was not significantly different in the CSU group (6.7 ng/mL; IQR 5.1-12.8) compared to the control group (9.6 ng/mL (4.9-28.3); p=0.377). Similarly, the median quantity of anti-FcεRI was not significantly different (52.4 ng/mL; IQR 25.7-126.5 vs. 49.3 ng/mL; IQR 29.8-177.1; p=0.649). The median avidity of anti-IgE was 75.8% (IQR 55.0-91.8), and the median avidity of anti-FcεRI was 75.0% (48.8; 90.1), which also did not differ from the control group (p=0.951 and p=0.425, respectively).

Next, we used the CU-BAT to investigate whether basophil activation differed between patients with CSU and healthy controls (Table I). In contrast to the quantity and affinity of autoantibodies, the median frequency of activated basophils was significantly higher in the CSU group compared to the control group (2.8% (IQR 1.2-4.9) vs. 0.7% (IQR 0.5-1.1), p<0.001). According to the literature, we set the cut-off for CU-BAT positivity at >7.6% as an indication of aiCSU (3). We observed a positive CU-BAT in 7 patients with CSU (14%) and none of the control group using this cut-off. To determine whether immunoglobulins are sufficient for basophil activation, we heat-inactivated the serum to degrade all heat-labile factors and preserve immunoglobulins. Both the frequency of activated basophils and subjects with a positive CU-BAT remained significantly higher in patients with CSU, indicating that antibodies are required for the enhanced basophil activation seen in patients with CSU.

Comparison of autoantibodies and basophil activation in patients with CSU at baseline and 6 months

To determine whether autoantibodies change over time in patients with CSU, we compared the quantity and avidity of autoantibodies and frequency of basophil activation at baseline and the 6-month follow-up. At 6 months, the median quantity of anti-IgE increased from 6.7 ng/mL (IQR 5.1-12.5) to 23.8 ng/mL (IQR 12.3-121.5; p<0.001), and the quantity of anti-FcεRI increased from 52.4 ng/mL (IQR 26.3-11.4) to 129.5 ng/mL (IQR 73.7-253.7; p<0.001) (Fig. 1). In contrast, the median avidity of anti-IgE decreased to 75.8% (IQR 55.3-90.7) to 56.4% (IQR 30.6-76.2; p=0.019), and the avidity of anti-FcεRI decreased from 75.1% (IQR 49.8-90.0) to 52.2% (IQR 38.2-60.1; p<0.001) after 6 months (Fig. 1). Interestingly, the median frequency of activated basophils did not differ significantly after 6 months (2.8% (IQR 1.2-4.9) to 1.8% (IQR 0.5-4.3; p=0.805) (Fig. 2). The frequency of CU-BAT-positive patients decreased after 6 months but was not statistically significant. We next compared the baseline and 6-month timepoints of patients with elevated levels of anti-IgE or anti-FcεRI autoantibodies (Table II). Patients were classified as having elevated autoantibodies if the autoantibody quantity was higher than the mean plus twice the standard deviation of the control group at baseline. In these patient subgroups, the quantity of anti-IgE and anti-FcεRI also increased while the avidity decreased. However, the difference in autoantibody quantity was not statistically significant.

We saw similar results in patients where CSU subsided after 6 months (Fig. S1). There were no significant differences in the frequency of activated basophils at baseline and 6 months in either subgroup (Table II).

Correlation of autoantibody avidity with the frequency of activated basophils

We next determined whether the frequency of activated basophils correlated with anti-IgE and anti-FcεRI avidity. Surprisingly, there was no significant correlation between the frequency of activated basophils and autoantibody avidity (Fig. 3). Similar results were seen in the subgroup of patients with elevated autoantibodies at baseline (data not shown).

CSU activity

CSU activity was calculated based on the UCT score. After 6 months, there was a significant increase, which means a lower CSU activity. The median UCT score at baseline was 6.0 (3.0; 8.0) and at 6 months 12.0 (11.0; 14.5), $p<0.001$.

Posthoc analysis

We evaluated a subset of study participants (11 CSU Patients and 6 healthy controls) at baseline with a second avidity assay by SPR. The avidity was measured by the equilibrium dissociation constant (KD). The median KD value at baseline for CSU patients was 0.170 [0.065; 0.336] M/1000000 and 0.029 [0.022; 0.232] M/1000000 for healthy controls, p=0.16. We compared the SPR avidity values to the ELISA avidity and calculated Spearman's rho. We found a moderate to a strong positive association between the two assays for the avidity of anti-IgE (Spearman's rho +0.65, p=0.031) and a strong association for the avidity of anti-FcεRI (Spearman's rho +0.73, p=0.014).

Subgroup analysis with exclusion of patients under omalizumab and with systemic steroids prior to inclusion

Even after exclusion of all patients on omalizumab and those on systemic steroids before study inclusion, there was an increase in the quantity of anti-IgE and anti-FcεRI and a decrease in avidity. We found no relevant differences of the primary endpoint after exclusion of these patients. Details of this subgroup analysis are shown in table III.

Discussion

In this study, we found that the quantity of anti-IgE and anti-FcεRI autoantibodies increased, while the avidity decreased over 6 months in patients with CSU, independent of disease course. In contrast, the frequency of activated basophils did not change over time. Unlike previous studies (4), we found signs of aiCSU (based on a positive CU-BAT) in only 14% of patients with CSU. Consistent with our study, MacGlashan et al. also demonstrated a lower frequency of aiCSU (24). Although aiCSU is associated with the presence of anti-FcεRI and anti-IgE autoantibodies, not all patients with a positive CU-BAT had elevated autoantibodies, and the quantity significantly changed over 6 months. Our data suggest that the amount of anti-FcεRI and anti-IgE autoantibodies is dependent on the time of analysis, does not correlate to disease activity and is therefore not suitable for the diagnosis of aiCSU.

Our data did not support our hypothesis of a correlation between autoantibody avidity and the frequency of activated basophils, not even in the subgroup analysis of patients with elevated autoantibodies at baseline. Interestingly, the quantity and avidity of both autoantibodies did not differ significantly between CSU patients and healthy controls at baseline. Healthy subjects may have non-functional anti-FcεRI belonging to the IgG2 subclass, which are unable to activate mast cells/basophils. (25) An additional explanation for this finding might be that autoantibodies play only a partial role in aiCSU and that a second signal is required (13). Indeed, numerous other factors like autoantibodies such as IgG anti-thyroperoxidase or IgE antibodies against autoantigens, which we did not measure in this study, and complement play an important role [26; 27]. Several studies have shown that the coagulation cascade as well as the complement system, in particular C5a is relevant in the pathogenesis of CSU, both as an augmentation factor related to autoantibodies, but also independently (27; 28; 29). Furthermore, we also performed the CU-BAT with heat-inactivated serum to determine the direct influence of immunoglobulins and did not observe a significant correlation. As the avidity measurement by ELISA with chaotropic reagents is not standardized in CSU and a novel approach, we compared a subset of baseline participants with an alternative approach by SPR and found for anti-IgE a moderate to strong and for the avidity of anti-FcεRI a strong association between the two assays.

The increase of autoantibody quantity and the simultaneous decrease of avidity could be attributed to excess and constant antigen exposure, which may impair further affinity maturation, resulting in an

enhanced number of low avidity antibodies as observed in patients with infectious diseases (30). Alternatively, there may be a trade-off between high affinity and stability (31). Interestingly, we observed the same autoantibody dynamics in patients with CSU treated with omalizumab, suggesting high disease activity and milder disease, including those with subsided CSU. Our data demonstrate a decrease in the UCT score after 6 months; however, this may have been influenced by medications and not a reflection of actual disease activity. We suspect that the autoantibody changes occur in all patients with CSU over time, regardless of disease progression. Interestingly, basophil activation does not appear to be dependent on this mechanism.

A weakness of our study is the inclusion of all CSU cases, regardless of phenotype. Only a fraction of our patients qualified as aiCSU based on a positive CU-BAT result or elevated autoantibodies, which hampered the assessment of autoantibody dynamics. However, the subgroup of study participants with autoantibody titers over the cut-off level showed similar results. In further studies, aiCSU patients should be pre-selected, e.g., with the inclusion of CU-BAT-positive individuals only.

The avidity evaluation in CSU by ELISA via chaotropic reagents is a new approach. To substantiate the results, we performed a posthoc analysis with SPR as a different avidity analysis to compare the results of aviditiy measurements. Another limitation is the assessment of the UCT score without considering individual drug intake. Drug therapy was very heterogeneous and changed significantly in most patients during the 6 month observation period, resulting in a significant reduction in the UCT. Therefore, a conclusive comparison of the UCT score to CU-BAT and antibody results was not possible. In addition, some patients received omalizumab treatment during the study and had short courses of systemic steroids prior to inclusion. Especially omalizumab influenced the analysis of anti-IgE antibodies. Therefore, we omitted the 6-month measurements of anti-IgE values in patients under omalizumab. An influence on other laboratory values (e.g. IgG values under systemic steroids) cannot be excluded. However, systemic steroids were used only for short courses with a dose of maximal 50mg prednisolone. In addition, omalizumab does not appear to have an effect on IgG (32). A subgroup analysis excluding these patients as well as those with a history of systemic steroids showed no significant changes of the primary endpoints.

Our study shows that aiCSU patients are significantly less frequent than expected. This may be related to a certain selection bias in previous studies, especially since severe and longtime CSU courses are increasingly treated in large central hospitals. In future, our findings should be examined exclusively in aiCSU or patients with elevated autoantibody titers. A more extended observation period and comparing the avidity with clinical activity (together with the recording of drug intake) would be important.

Conclusion

In this study, we found that in patients with CSU, the quantity of anti-FcεRI and anti-IgE autoantibodies increases, and the avidity decreases over time. However, neither the quantity nor the avidity of these autoantibodies correlates with disease course or the frequency of activated basophils, suggesting that factors independent of these two autoantibodies play a significant role in aiCSU. Our data suggest that the quantity and avidity of anti-FcεRI and anti-IgE autoantibodies are dependent on the time of analysis and are therefore not suitable for the diagnosis of aiCSU.

Compliance with Ethical Standards Statements

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Author contribution

LJ, NMW and OH designed and planned the study, LJ acquired funding, LJ, NMW, KK and OH acquired the data, LJ, NMW, KK WP, OS and OH analysed the data and participated in the interpretation of the data, LJ and OD performed the statistical analysis, LJ, NMW and OH wrote the manuscript. All authors critically reviewed the manuscript and gave final approval of the submitted work.

Conflicts of interest

NMW, KK, WP and OH are employees of ADR-AC GmbH, a specialized laboratory offering basophil activation tests for routine diagnostics in Switzerland.

Data Availability Statement

The data that support the findings of this study will be available from the corresponding author upon reasonable request.

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Table I - Patient characteristics and comparison of CSU patients and controls at baseline.

	All	**Patient**	**Control**	**p-value**
	n (%) or median (IQ-range)	n (%) or median (IQ-range)	n (%) or median (IQ-range)	
Total N	N = 79	N = 49	N = 30	
Gender (male)	24 (30%)	14 (29%)	10 (33%)	0.655
Age (years)	31.0 (24.0; 48.0)	35.0 (25.0; 49.0)	26.5 (23.0; 39.5)	0.050
Duration of disease (months)	31.0 (24.0; 48.0)	7.0 (4.0; 15.0)	-	-
Angioedema (yes)	31.0 (24.0; 48.0)	28 (57%)	-	-
Antihistamines (yes)	31.0 (24.0; 48.0)	46 (94%)	-	-
Montelukast (yes)	31.0 (24.0; 48.0)	2 (4%)	-	-
Corticosteroids (yes)	31.0 (24.0; 48.0)	5 (10%)	-	-
Omalizumab (yes)	31.0 (24.0; 48.0)	1 (2%)	-	-
UCT	10.0 (6.0; 16.0)	6.0 (3.0; 8.0)	16.0 (16.0; 16.0)	<0.001
anti-IgE Quantity ng/mL	7.5 (5.0; 13.5)	6.7 (5.1; 12.8)	9.6 (4.9; 28.3)	0.377
anti-IgE Avidity %	72.7 (51.9; 92.9)	75.8 (55.0; 91.8)	68.1 (49.1; 94.7)	0.951
anti-FcεRI Quantity ng/mL	50.0 (26.8; 141.6)	52.4 (25.7; 126.5)	49.3 (29.8; 177.1)	0.649
anti-FcεRI Avidity %	76.2 (53.3; 90.2)	75.0 (48.8; 90.1)	79.6 (54.7; 91.2)	0.425
CU-BAT	1.3 (0.6; 3.8)	2.8 (1.2; 4.9)	0.7 (0.5; 1.1)	<0.001
CU-BAT heated	2.8 (1.9; 3.6)	3.0 (2.0; 4.4)	2.4 (1.5; 3.2)	0.008
CU-BAT >7.6% (yes)	7 (9%)	7 (14%)	0 (0%)	0.040
CU-BAT heated >7.6% (yes)	10 (13%)	10 (20%)	0 (0%)	0.011
anti-IgE Quantity >control mean+2sd	18 (23%)	8 (16%)	10 (33%)	0.104
anti-FcεRI Quantity >control mean+2sd	24 (30%)	15 (31%)	9 (30%)	1.000

Table II - Subgroup analysis of patients with elevated levels of anti-FcεRI (n=9) and anti-IgE (n=5) at baseline.

	Baseline	**6 months**	
	median (IQ-range)	median (IQ-range)	p-value
anti-IgE Quantity subgroup			
anti-IgE Quantity (ng/mL)	86.5 (30.5 ; 293.3)	1078.8 (34.4 ; 1215.7)	0.138
anti-IgE Avidity %	22.6 (9.7 ; 44.5)	7.4 (3.7 ; 12.7)	0.043
CU-BAT	1.5 (0.9 ; 3.1)	2.8 (1.5 ; 5.3)	0.463
anti-FcεRI Quantity subgroup			
anti-FcεRI Quantity (ng/mL)	151.6 (141.6 ; 499.4)	260.8 (162.4 ; 464.8)	0.173
anti-FcεRI Avidity %	45.9 (20.4 ; 51.2)	32.6 (17.9 ; 39.1)	0.051
CU-BAT	2.5 (1.2 ; 5.6)	1.8 (0.3 ; 5.3)	0.594

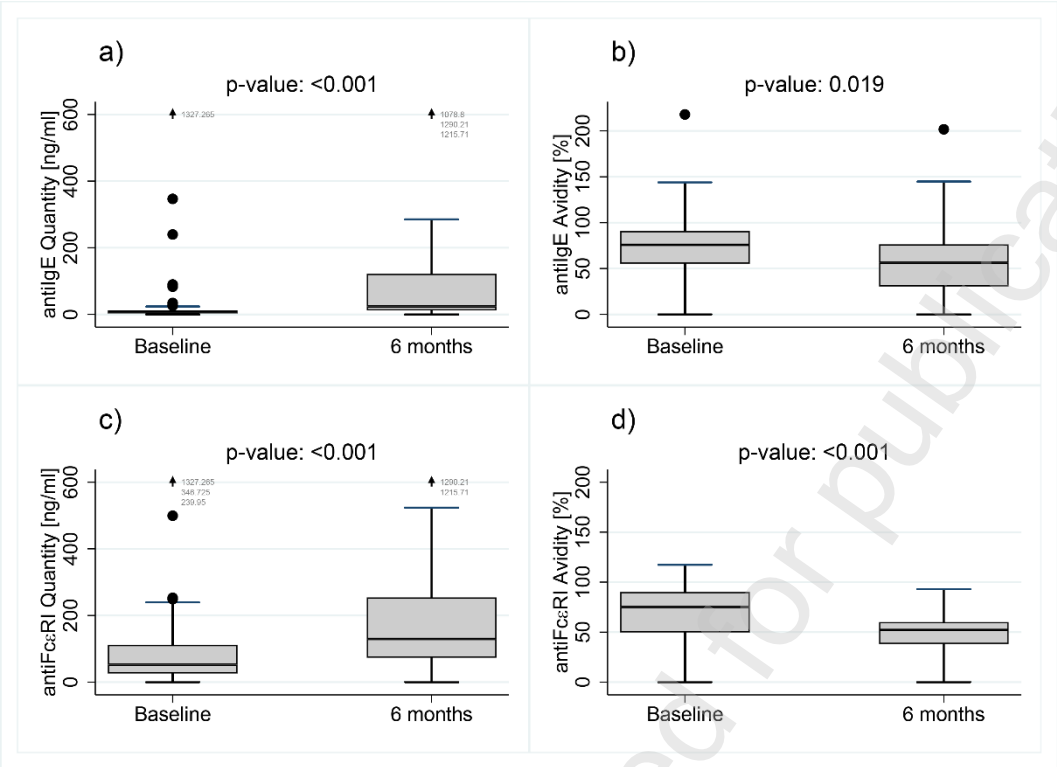
All patients with elevated autoantibody titers at baseline were analyzed independently for subgroup analysis. Cut-offs for subgroup analysis were determined as the mean plus twice the standard deviation of the control group. P-values were calculated by the Wilcoxon signed-rank test.

Table III - Subgroup analysis of patients without omalizumab or systemic steroids (n=21).

	Baseline	**6 months**	
	median (IQ-range)	median (IQ-range)	p-value
anti-IgE Quantity subgroup			
anti-IgE Quantity (ng/mL)	7.1 (5.3 ; 12.4)	25.6 (12.3 ; 121.5)	<0.001
anti-IgE Avidity %	76.1 (54.7 ; 88.7)	53.4 (30.6 ; 76.2)	0.027
anti-FcεRI Quantity subgroup			
anti-FcεRI Quantity (ng/mL)	68.7 (26.7 ; 143.0)	153.4 (83.8 ; 260.8)	0.002
anti-FcεRI Avidity %	75.2 (47.7 ; 90.2)	53.6 (38.2 ; 62.7)	0.001
CU-BAT	2.4 (0.8 ; 4.7)	2.8 (1.0 ; 5.1)	0.198

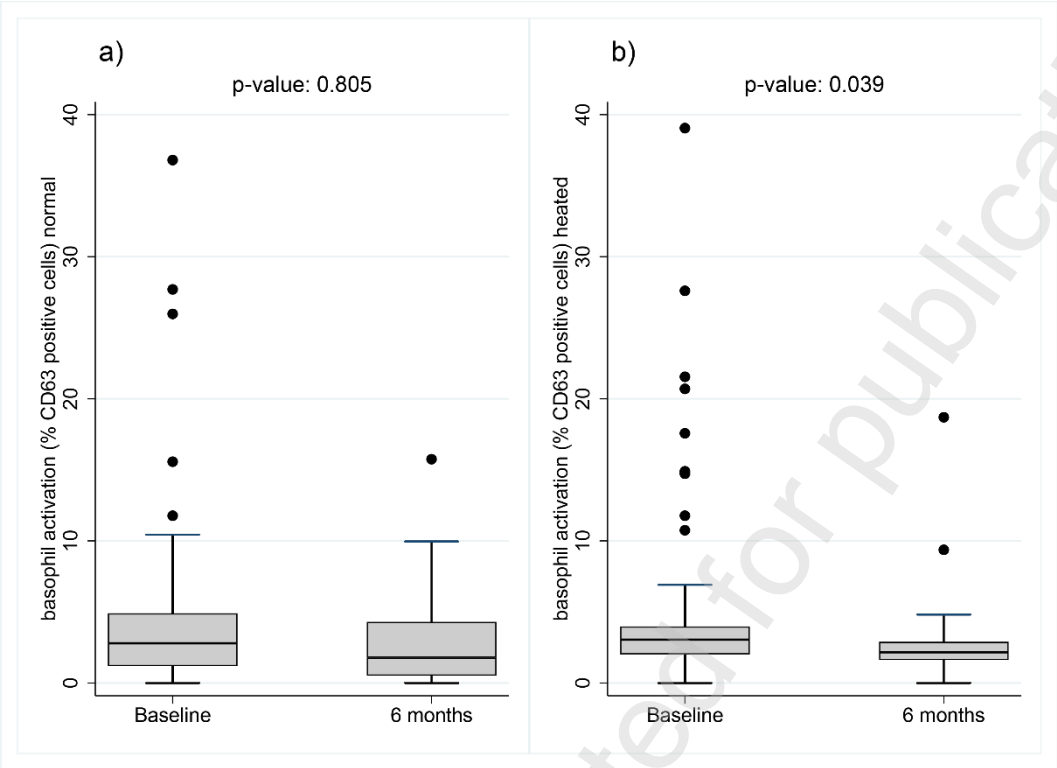
IgG antibodies against FcεRIα and IgE were evaluated for quantity and avidity using an ELISA at baseline and 6 months. Data represent the median and interquartile range at each timepoint. P-values were determined using a Wilcoxon signed-rank test. Only the patients with baseline and 6 months measurements were considered for the computation.

Figure 1 - Quantity and avidity of anti-FcεRI (n=30) and anti-IgE autoantibodies (n=22) in patients with CSU at baseline and the 6-month follow-up.



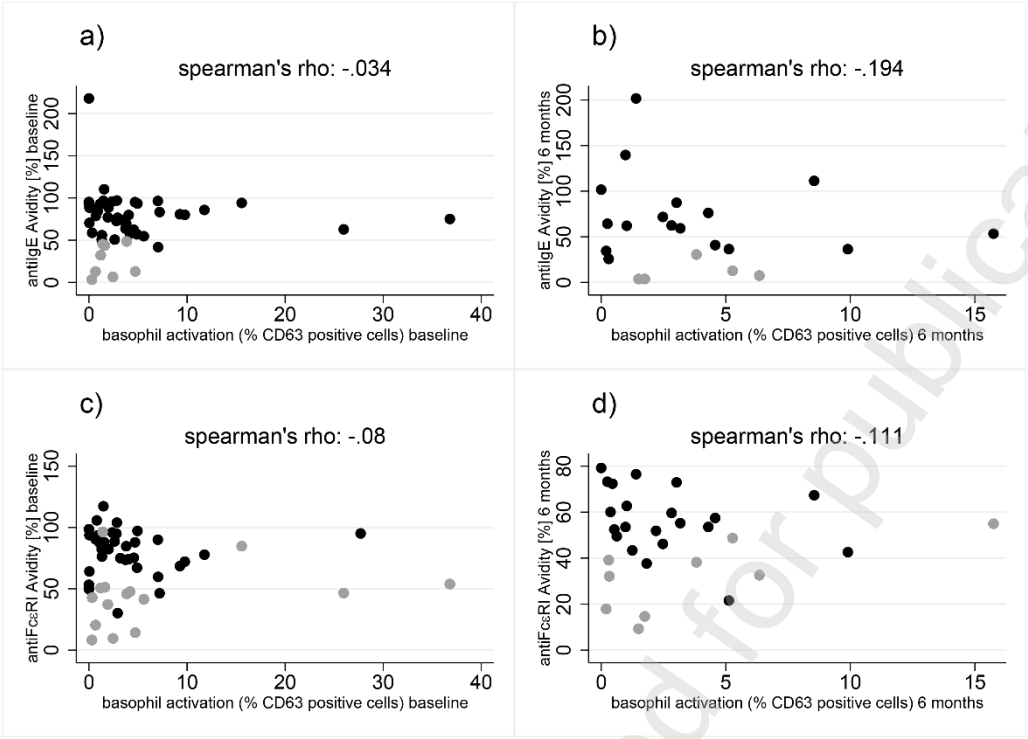
IgG antibodies against FcεRIα and IgE were evaluated for quantity (a, c) and avidity (b, d) using an ELISA at baseline and 6 months. Data represent the median and interquartile range at each timepoint. P-values were determined using a Wilcoxon signed-rank test. Outliers which lie out of the graph range are indicated with an arrow. Only the patients with baseline and 6 months measurements were considered for the computation.

Figure 2 - The frequency of activated basophils in patients with CSU at baseline and the 6-month follow-up (n=30).



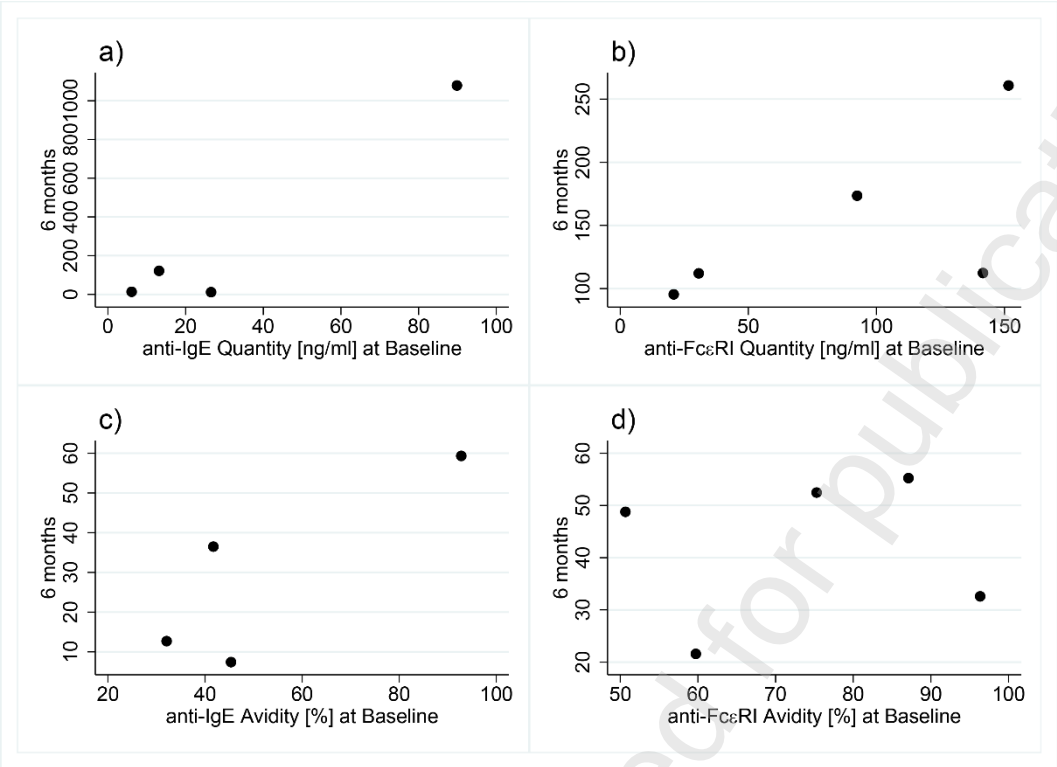
The serum of patients with CSU at baseline and 6 months were incubated with basophils isolated from 2 healthy donors (CU-BAT) to determine the presence of activating serum factors (a). CU-BAT analysis of heat-inactivated serum (b). CD63 served as an activation marker and was expressed as the percentage of activated basophils (mean of two measurements). Data represent the median and interquartile range at each timepoint. P-values were determined using a Wilcoxon signed-rank test.

Figure 3 - Correlation of the frequency of activated basophils with the avidity of anti-FcεRI and anti-IgE autoantibodies in patients with CSU.



Association of the frequency of activated basophils, as determined by a basophil activation test, and the avidity of anti-IgE (a; n=48, b; n=22) and anti-FcεRI (c; n=49, d; n=30) autoantibodies (%) at baseline and 6 months. The strength of association was calculated by Spearman's rho. Patients with autoantibody quantities above the cut-off value at baseline are plotted in grey.

S1 Figure - Quantity and avidity of anti-FcεRI (n=5) and anti-IgE autoantibodies (n=4) in patients with subsided CSU at 6 months.



Individual course of the quantity and avidity of anti-FcεRI and anti-IgE autoantibodies in patients with subsided CSU after 6 months.