Double-blind placebo-controlled trial of the effect of omalizumab on basophils in chronic urticaria patients

Short Title: Effect of omalizumab on basophils in chronic urticaria patients

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Abstract:

Background: Omalizumab has been shown to be effective in treating chronic spontaneous urticaria (CSU). The reduction of FcεRI receptor density on the surface of basophils and mast cells is thought to play a major role in its effectiveness. We conducted a double-blind, randomized, placebo-controlled trial to investigate the mode of action of omalizumab in patients with antihistamine-resistant CSU.

Methods: Thirty patients were randomized in a 2:1 ratio to receive either 300 mg omalizumab or placebo. Four monthly applications of omalizumab/placebo were followed up with a visit 2 months after the last injection. The primary endpoint was the FcεRI receptor density change on basophils.

Results: Omalizumab led to a significant reduction of FcεRI receptor density on basophils as soon as 1 week after the first injection: baseline omalizumab vs. placebo group, 80.31 ± 47.18x10^3 vs. 78.29 ± 45.09x10^3 receptors/basophil ± SD; 1 week, 72.89 ± 47.79x10^3 vs. 27.83 ± 20.87x10^3, p=0.001. This effect continued during the treatment phase and persisted for 2 months after the last injection: 93.81 ± 56.50x10^3 vs. 21.09 ± 15.23x10^3, p=0.002. Values for basophil "releasability" and the basophil activation test (CU-BAT) of patient serum using donor basophils were unchanged despite treatment: CU-BAT, CD63 10.75% (7.35) in the placebo group vs. 8.35% (15.20) in the omalizumab group, p=0.778.

Conclusion: We demonstrated a rapid reduction of FcεRI receptor density on basophils following treatment with omalizumab. Because CU-BAT using well-characterized, omalizumab-naïve donor basophils did not change during the treatment phase, autoreactive serum factors seem to remain unaltered. This points towards a cellular effect of omalizumab on basophils. To predict the omalizumab response time and to monitor disease, FcεRI density and CU-BAT might be promising cellular-based assays.

Introduction

Chronic spontaneous urticaria (CSU) is a common disease with a point prevalence of 1% of the general population [1]. Patients manifest with hives on most days for a period of at least 6 weeks. The disease is often accompanied by angioedema and tends to be cyclical in nature, with spontaneous disappearances and frequent relapses [2]. In most cases, no underlying disease or allergy can be found [3].

CSU treatment relies mainly on second-generation antihistamines and is purely symptomatic. While some patients respond to standard-dose second-generation antihistamines, in others off-label (for CSU) treatments with high-dose H1-antihistamines, leukotriene receptor blocking agents, cyclosporine A, or systemic corticosteroids are needed [4, 5].

Based on several randomized controlled trials [6 – 8], the monoclonal anti-IgE antibody omalizumab was recently licensed for the treatment of antihistamine-resistant CSU [9]. The mechanism of action of omalizumab in CSU is still not fully understood. Omalizumab binds selectively to free IgE in plasma, inhibits its binding to FcεRI receptors on the surface of mast cells and basophils, and reduces the number of FcεRI receptors on basophils in atopic patients [10, 11] and those with CSU [12, 13]. A significant reduction of FcεRI receptor density on the surface of circulating basophils has been found as early as 1 week after the administration of omalizumab [14]. In contrast, a decrease in FcεRI receptor density on mast cells may occur later [11]. The role of basophils in CSU is still debated [15], but basophils may be an accessible and relevant model to analyze the effect of omalizumab on FcεRI expression and on the level of basophil activation.

About half of CSU patients have autoantibodies against FcεRI, few against IgE [16, 17]. Other autoimmune markers such as IgE and IgG antibodies against thyroid peroxidase are frequently found [18]. Sera of CSU patients can activate the resting basophils of normal donors to release histamine and upregulate the activation markers CD63 and CD203c [19, 20]. The basophil activation test with donor basophils (CU-BAT) involves measuring CD63 upregulation as an activation marker and is already established as a specific, sensitive, and safe in vitro assay to detect functional autoantibodies [19 – 22]. In addition, the FcεRI receptor density itself might be another important parameter for the quantification of mast cell and basophil “releasability” and therefore a good in vitro surrogate marker for their reactivity.

To gain insight into the pathophysiology of some forms of CSU and the mode of action of omalizumab, we evaluated the effect of omalizumab treatment in patients with CSU by

investigating three parameters: FcεRI receptor density on the surface of basophils, determined by flow cytometry; the functional consequences of a change in FcεRI receptor density, evaluated via the crosslinking of FcεRI / IgE receptor complexes induced by autoantibodies (basophil releasibility); and whether typical CSU serum factors responsible for basophil degranulation were altered during treatment. Based on the findings of Gericke et al. [23], a post hoc analysis was performed to identify differences between early and slow responses to omalizumab.

Methods

Study design

In a monocentric, double-blind, randomized, placebo-controlled trial we investigated the mechanism of action of omalizumab in antihistamine-resistant CSU over a period of 7 months, between September 2012 and April 2014. Thirty patients diagnosed with CSU were recruited and randomly assigned to two groups in a 2:1 ratio to receive either omalizumab (n=20) or placebo (n=10). Omalizumab (Xolair®) was administered in four monthly treatments at a fixed subcutaneous dose of 300 mg as currently licensed [6, 24]. Before the first administration, disease activity was assessed and documented over a 4-week period with the urticaria activity score (UAS), followed by the 16-week treatment period with 4 injections of omalizumab/placebo. Two months after the last injection, a final study visit to follow up treatment efficacy and study outcomes took place.

The study was approved by the local ethics committee and by Swissmedic, the Swiss agency for therapeutic products. The trial was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation (ICH)/WHO Good Clinical Practice, and all national, state, and local laws.

This investigator-initiated study was funded by Novartis Pharmaceuticals (Basel, Switzerland). Their representatives were not involved in the design of the study or any data collection, analysis, or interpretation.

Study population

Patients between the ages of 18 and 70 with CSU were included in the study if they met the following criteria: symptoms for at least 6 weeks, with hives present at least twice weekly, and symptoms refractory to standard doses of H1-antihistamines at the time of randomization. Patients signed informed consent forms documenting their understanding of the study procedures and the investigational nature of the study. Exclusion criteria were purely inducible urticaria, clearly defined allergic urticaria, treatment with omalizumab during the last year, known hypersensitivity to omalizumab or any of its components, history of cancer in the previous 5 years, parasitic infections, documented active tuberculosis or ongoing recent (in the preceding 4 weeks) antituberculous therapy, documented prior treatment with systemic immunosuppressive agents (short-term prednisolone for CSU exacerbations was allowed), current pregnancy or nursing, known intolerance to any protocol intervention, and lack of accountability. Any contraception had to be performed by a safe,

reliable, and accepted method such as oral or implanted contraceptives or tubal ligation. Patients were allowed to take their usual medications (H1-antihistamines up to a fourfold single dose, H2-antihistamines, montelukast, short-term prednisolone for CSU exacerbations) for the treatment of CSU, but systemic immunosuppressive agents other than prednisolone were not permitted, as they might influence basophil assays.

Basophil assays

Two assays evaluated basophil phenotype and function, while another measured the presence of serum factors inducing donor basophil degranulation/activation.

a) The FcεRI density on basophils was measured at randomization and on the day of the first treatment, and thereafter at 1 week, 1 and 3 months, and 2 months after stopping treatment. The FcεRI receptor density was assessed by QIFIKIT (Dako-Cytomation, Glostrup, Denmark) according to the instructions of the manufacturer [25]. For each quantification, 500-1000 basophils were acquired using CCR3 basophil-specific staining. FcεRI receptor density was defined according to the standard curve using beads with ascending predefined receptor densities.
b) Basophil releasability was measured with and without IL-3 coincubation at three timepoints (baseline, 1 week, and 3 months after the first injection) by assessing the activation marker CD63 [26] after stimulation with anti-IgE (not omalizumab, Beckman Coulter, Krefeld Deutschland) at the concentrations of 1000, 100, 10 and 1ng/ml. Cells were stained with monoclonal anti-CD63-FITC antibodies (BioLegend, San Diego, CA, USA) and fluorescence was measured by FACSCanto® (Becton Dickinson AG, Allschwil, Switzerland)
c) The CU-BAT involved incubating the serum of study participants on well-characterized basophils from 10 donors both at baseline and at the last injection to detect the presence of activating serum factors. CD63 served as an activation marker, with expression data presented as the percentage of activated basophils and the stimulation index (SI), expressed as the percentage of activated basophils after stimulation with patient serum divided by the percentage of activated basophils without stimulation (negative control) [22]. The donors were previously screened for reactivity to negative and positive controls as well as for basophil activation with different IL-3 concentrations. All serum samples were immediately stored at -20°C in aliquots to avoid repetitive freeze-thaw cycles.

Patient assessments

All participants were required to document their symptoms once daily for 1 month using the urticaria activity score [27]. Medications taken were documented on their study questionnaires.

Study endpoints

The primary endpoint of the study was the FcεRI receptor density change on basophils during the first 12 weeks of treatment with omalizumab and 2 months after treatment.

Secondary endpoints included comparison of basophil releasibility with and without IL-3 incubation at three timepoints (baseline, 1 week, and 3 months); comparison of CU-BAT at baseline and 3 months; and change in the weekly urticaria activity score (UAS7) at baseline, 3 months and 5 months. We evaluated the proportion of patients with a UAS7 of ≤6, a UAS7 of 0, and an itch severity score (ISS) of 0.

We summarized the proportion of patients who used H1- and H2-antihistamines, montelukast and prednisolone, and coded adverse events using the medical dictionary for regulatory activities (MeDRA) and their corresponding preferred terms and assigned body systems.

Post hoc analysis

Recently, Gericke et al. observed that the presence of autoreactive serum components predicted the response time to omalizumab treatment [23]. We therefore performed a post hoc analysis to identify differences in FcεRI-receptor density, autoreactive serum components (measured by CU-BAT), and total IgE in omalizumab partial/non-responders, early responders, and slow responders. Based on a previous report [23] we divided all study patients into 4 groups: placebo; partial/non-responder, defined as a UAS7 >6 at 3 months or a reduction of UAS7 to ≤6 or less at 3 months compared to baseline; early responder, defined as a treatment response within 8 days, with the first day of response determined as the first day of UAS7 <6; and slow responder, defined as a treatment response after 8 days but UAS7 <6 at 3 months. For each of these groups, we compared FcεRI receptor density, CU-BAT results, and total IgE at baseline, and calculated whether there was a difference in FcεRI density and CU-BAT between baseline and 3 months.

Sample size determination

A biological effect for the primary outcome was determined to be a difference of FcεRI receptor density of 45,000 per basophil, assuming a standard deviation of 40,000 Fc-IgE per

basophil. A power of 80%, a two-sided p-value of 0.05, and 2:1 randomization resulted in an overall sample size of 30 patients, with 20 in the active group and 10 in the placebo group.

Analysis of primary, secondary, and other efficacy endpoints

The primary endpoint, FcεRI receptor density, was analyzed using generalized estimation equations (GEE) with a negative binomial distribution family, log link, and robust standard errors due to strong skewness and heteroscedasticity when using mixed effects linear models. Treatment group and baseline receptor density were included as fixed effects. The mean of the two pretreatment visits was taken as the baseline receptor density. We report the ratio between treatments, with a ratio <1 indicating a reduction in receptor density. We used the same methods to analyze FcεRI receptor density at each follow-up time.

Analysis of the primary endpoint followed the intention-to-treat (ITT) principle. Missing primary endpoint values were imputed by multiple imputation techniques using sequential chained equations based on baseline characteristics. Altogether, 12 of 150 (8.0%) FcεRI receptor measurements were missing, the majority at the last visit.

As a sensitivity analysis, we also performed the main analyses on the per-protocol population. Twenty-four patients who completed all treatments according to the protocol were included.

Secondary endpoints are summarized using descriptive statistics. Odds ratios (OR) for the UAS7 and ISS were calculated using penalized logistic regression by Firth's method [28].

Analysis was performed using Stata 13.1 and 14 [29]. For all analyses we report estimates and 95% confidence intervals (CI) when relevant. Descriptive statistics report mean and standard deviation (SD) for continuous and normally distributed data or median and interquartile range (IQR) for non-normally distributed or highly skewed data. Count and percentages are reported for binary data.

Results

Study Patients

A total of 77 patients were screened, 41 of whom were excluded, 32 for good disease control with antihistamines, 4 for use of immunosuppressive drugs, 4 for spontaneous remission, and 1 for use of omalizumab in the last 12 months. Six patients declined to participate. Two patients in the placebo group withdrew consent as a consequence of disease progression, and two in the omalizumab group were lost to follow-up (Figure 1).

Most baseline characteristics (age, weight, BMI) did not differ between the placebo and omalizumab groups (Table 1). The proportion of women was 80% in the placebo group and only 40% in the omalizumab group, but probably because of the small sample size. The UAS7 at baseline was 18.5 (11.3, 23.5) in the placebo group and 11.0 (2.5, 21.5) among omalizumab patients, while the ISS at baseline was the same in the two groups. Angioedema occurred in 53% of patients (70% in the placebo group, 45% in the omalizumab group). Disease duration was 27.0 (19.8, 64.8) months in the placebo group and 19.5 (12.0 33.8) in the omalizumab group.

There were eight partial/non-responders, seven early responders, and two slow responders in the omalizumab group; three patients who dropped out could not be classified. There were no partial responders.

FcεRI receptor density

At baseline, no significant differences in FcεRI receptor density were found between the two groups (Figure 2, Table 6). Compared to placebo, 300 mg of omalizumab led to a rapid and significant reduction of FcεRI receptor density as soon as 1 week after the first injection ($72.89 \pm 47.79 \times 10^3$ vs. $27.83 \pm 20.87 \times 10^3$ receptors/basophil, respectively, p=0.001). Again compared to placebo, the receptor density in the omalizumab group remained low throughout the treatment period (values at 3 months: $95.88 \pm 54.17 \times 10^3$ vs. $17.47 \pm 21.41 \times 10^3$, respectively, p=<0.001), and was still low at 2 months after the last injection ($93.81 \pm 56.50 \times 10^3$ vs. $21.09 \pm 15.23 \times 10^3$, respectively, p=0.002).

Basophil releasability

Regarding basophil releasability, expressed as Log10 of the anti-IgE (not omalizumab) concentration resulting in half-maximal activation of basophils (LC50), no significant difference of the LC50 value could be found between the two groups at the start of treatment (with or wthout IL-3). Treatment did not result in a consistent change of releasibility (Table

2a). Interestingly, the basophil releasability measured with IL-3 coincubation decreased in the omalizumab group during the study (higher values indicate lower basophil releasability). However, due to the small sample size, statistical significance cannot be shown.

Effect of omalizumab on serum factors

Patient serum was incubated twice with third-party donor basophils (at baseline and at 3 months). Compared to placebo, the percentage of activated donor basophils was slightly higher in the omalizumab group at baseline: the CD63 positivity was 12.05% (10.80) vs. 18.35% (17.77), respectively, p=0.244; the SI values were similar, 4.20 (5.50) vs. 5.00 (7.20), respectively, p=0.535. There was no significant difference in either parameter between the placebo and omalizumab groups, both at baseline and the last injection: CD63 positivity, 10.75% (7.35) vs. 8.35% (15.20), respectively, p=0.778; SI, 3.20 (2.45) vs. 2.75 (3.50), respectively, p=0.847. Treatment did not significantly affect the activity of these serum factors (Table 2b).

CSU activity

The UAS7 was summed for a week before each study visit, and the proportions of patients with mild (UAS7 of ≤6) or no symptoms (UAS7=0) were evaluated. The proportion of patients with no itching (ISS=0) in the week before the corresponding visit was also assessed (Table 3).

We observed no significant differences at baseline in UAS7≤6 (OR 1.47; 0.26, 8.43) or ISS=0 (1.21; 0.15, 9.92) between the placebo and omalizumab groups. We observed a significant difference in the proportion of patients with mild symptoms (UAS7 <=6) at the time of the last injection (7.80; 1.28, 47.53); it was increased in the omalizumab group (76%) but remained unchanged in the placebo group (25%). The proportion of patients with ISS=0 showed no significant difference between the two groups; the odds of having a score of 0 were higher in the omalizumab group, though the difference was not statistically significant (both 15.21; 0.76, 305.06). Two months after the last injection, the proportion of patients with UAS7≤6 and ISS=0 showed no significant difference between the placebo and omalizumab groups.

Post hoc analysis

In a post hoc analysis we classified all study patients into 4 groups: placebo, partial/non-responder, early responder, and slow responder (Table 4). At baseline, FcεRI density was equal among the placebo, slow responder, and early responder groups, but lower in the partial/non-responder group (p=0.094). Compared to placebo, there was a reduction of the

FcεRI density on basophils in all three treatment groups, although the partial/non-responder group demonstrated the least reduction: -45.3x10³ (-74.9; 0.3) receptors per basophil in the partial/non-responder group, -99.7x10³ (-138.9; -60.5) in the slow responder group, and -91.7x10³ (-154.3; -63.7) in the early responder group). As a sign of autoreactive serum components at baseline, CU-BAT results in the omalizumab group were highest in the partial/non-responder group (CD63 positivity 26.3% (8.7; 44.6)) and lowest in the early responder group (CD63 positivity 17.1% (4.5; 24.8)), though these differences were not statistically significant. There was a slight but nonsignificant reduction of the CU-BAT in all three treatment groups. Two patients in the partial/non-responder group had an increase of CU-BAT under omalizumab treatment. The total IgE values at baseline were lowest in the partial/non-responder group.

Discussion

We found that treatment with omalizumab resulted in a rapid and significant reduction of FcεRI density on basophils, which is consistent with previous studies [10, 11, 14] and confirms the results of Deza et al. [12] and Metz et al. [13]. This reduction was observed as soon as 1 week after the initiation of treatment and remained reduced for at least 2 months after the last omalizumab application (last study visit), although CSU often flares up by this later timepoint. The reduction of FcεRI density is thought to be one of the central mechanisms of omalizumab's action in CSU [30]. In accordance with recent findings [12], FcεRI density at baseline was reduced in patients who did not respond to this therapy. The partial/non-responder group demonstrated the least reduction of the FcεRI density under omalizumab treatment. Correspondingly, post hoc analysis showed that total IgE values before treatment were lowest in partial/non-responders.

We expected a reduction of basophil releasability, applying titrated concentrations of degranulating anti-IgE (not omalizumab) to patient basophils. However, this was not the case. Investigation of basophil releasability using a high-affinity degranulating anti-IgE antibody, even in titrated form, might produce a signal too intense to show subtle differences in basophil releasability. Basophil stimulation with an allergen (in sensitized individuals) might have led to different results. Unfortunately, the study included only nine pollen-sensitized patients, which was too few to confirm this hypothesis. Interestingly, treatment with omalizumab has been shown to lead to increased sensitivity of basophils using IgE-mediated stimuli [31], a phenomenon that is not fully understood. In addition, no alteration of mast cell or basophil histamine release was observed when cells were coincubated with omalizumab in vitro [32], but this might not reflect the in vivo activity of omalizumab.

We investigated changes of autoreactive serum factors, which may occur in CSU patients, during omalizumab treatment. By coincubating well-characterized donor basophils with the serum of CSU patients (CU-BAT), the omalizumab-treated basophils of the patient are replaced by treatment-naïve donor basophils, which allows a focus on transferable serum factors alone. Again, no difference was found between the two study groups either at baseline or over time. This suggests that omalizumab does not interact with or alter autoreactive or other urticaria-promoting serum factors. However, CU-BAT results were very heterogeneous and the sample size was small, which hampered the statistical analysis. Interestingly, higher CU-BAT results at baseline were associated with slow or partial/non-response to omalizumab, with partial/non-responders having the highest values, findings that

are consistent with those of Gericke et al. [23]. Two partial/non-responders even demonstrated an increase in CU-BAT under omalizumab treatment.

While CSU is a mast cell-driven disease, our experimental approach relied on basophils due to their better accessibility and the assumptions that a) basophils play a relevant role in the pathogenesis of CSU [13, 20], and b) analysis of basophil activity is a relevant model for changes of omalizumab-induced mast cell activity. While omalizumab does not lead to a significant reduction of autoreactive serum components, its effect might rely on cellular changes. It is well known that circulating basophils are recruited into skin lesions in CSU [33 - 35]. It is possible that omalizumab decreases the ability of basophils to migrate into the skin, for example by altering their chemokine receptor repertoire. Consistent with this hypothesis, Metz et al. showed a decrease in the number of FcεRI+ skin cells under omalizumab treatment [13]

Our study population was heterogeneous: 6 of our 30 patients had a changing disease course with only mild symptoms at baseline compared to the time of study inclusion. This reflects the natural course of CSU, which is characterized by intermittent remissions, and might explain the nonsignificant differences in various endpoints. Furthermore, as we intended to study patients with severe forms of CSU, there were eight partial/non-responders. This probably influenced the study outcomes, but gave us the opportunity to identify differences between slow, early, and partial/non-responders.

CSU tends to remit spontaneously, and thus far no sustained disease-modifying effect of omalizumab has been documented after the medication is discontinued. Furthermore, the response time after initiating omalizumab treatment varies greatly. FcεRI density on basophils, CU-BAT, and total IgE might be appropriate parameters to predict response time and disease course under omalizumab. Assessing these factors before initiating treatment with omalizumab could help differentiate between early, slow, and partial/non-responders. In particular, low FcεRI density and/or high CU-BAT at baseline might predict whether a patient will be a partial/non-responder. Practically speaking, it would be even easier to determine the total IgE value at baseline, as it corresponds to FcεRI density, meaning that a low total IgE predicts a low FcεRI density.
In patients who respond to omalizumab, treatment discontinuation is necessary to reveal the underlying disease status. As omalizumab did not lead to a reduction of CU-BAT, a disappearance of autoreactive serum components under omalizumab treatment might indicate remission of CSU. However, this assumption would only be valid in CU-BAT-positive CSU patients (approximately 30-40% of all CSU patients).

Conclusion

This study contributes to understanding CSU pathogenesis and the mode of action of omalizumab. It substantiates the previous observation that the reduction of FcεRI density is rapid and sustained. The clinical efficacy of omalizumab did not correlate with serum changes (CU-BAT results remained stable throughout the study period). This points towards a primarily cellular effect of omalizumab on basophils. To predict the response time of omalizumab and for disease monitoring, FcεRI density and CU-BAT are promising cellular-based tests if used before treatment initiation.

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Figures and tables

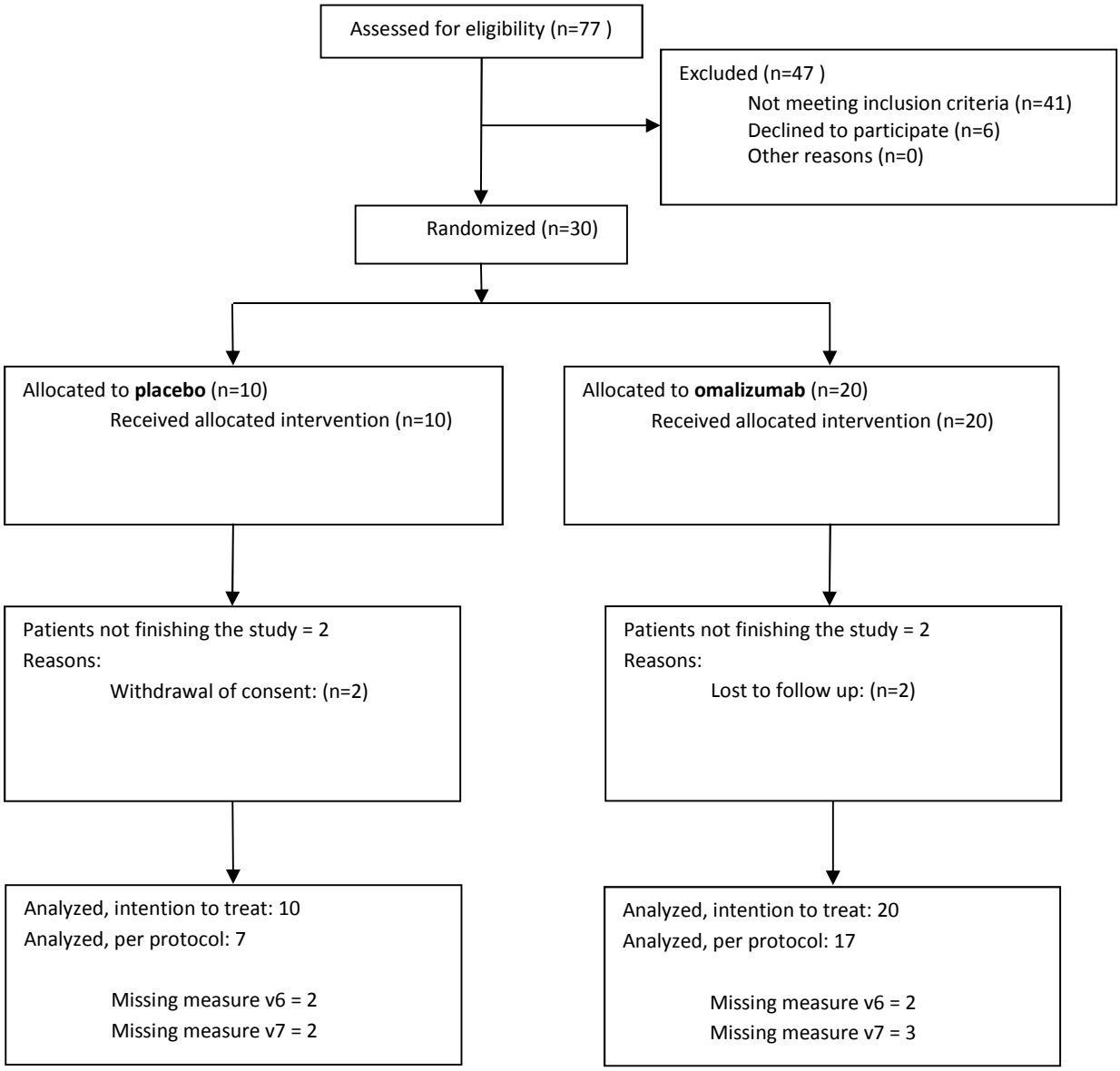

Figure 1 Enrollment and allocation

Table 1 Baseline characteristics

	Placebo N=10	Omalizumab N=20	p Value
Demographics			
Age (at inclusion)	42.4 ± 13.3	41.8 ± 15.2	0.915
Gender (female)	8 (80%)	8 (40%)	0.058
Weight (kg)	76.9 ± 16.9	83.6 ± 20.1	0.375
Height (cm)	164.7 ± 8.3	174.1 ± 8.1	0.006
Body mass index (kg/m2)	28.3 ± 6.0	27.5 ± 7.1	0.764
Clinical			
Duration of disease (months)	27.0 (19.8; 64.8)	19.5 (12.0; 33.8)	0.180
Angioedema (yes)	7 (70%)	9 (45%)	0.433
Urticaria activity score for 1 week (UAS7) at baseline	18.5 (11.3; 23.5)	11.0 (2.5; 21.5)	0.187
Itch severity score at baseline	8.0 (1.8; 12.0)	8.0 (1.8; 13.5)	1.000
Allergy to birch pollen (Yes)	2 (20%)	5 (25%)	1.000
Allergy to grass pollen (Yes)	4 (40%)	4 (20%)	0.384
Study complete (Yes)	8 (80%)	18 (90%)	0.584

Values are mean ± standard deviation for continuous variables, or median and interquartile ranges (IQR) for non-normally distributed or highly skewed variables. Categorical variables reported as n (%).

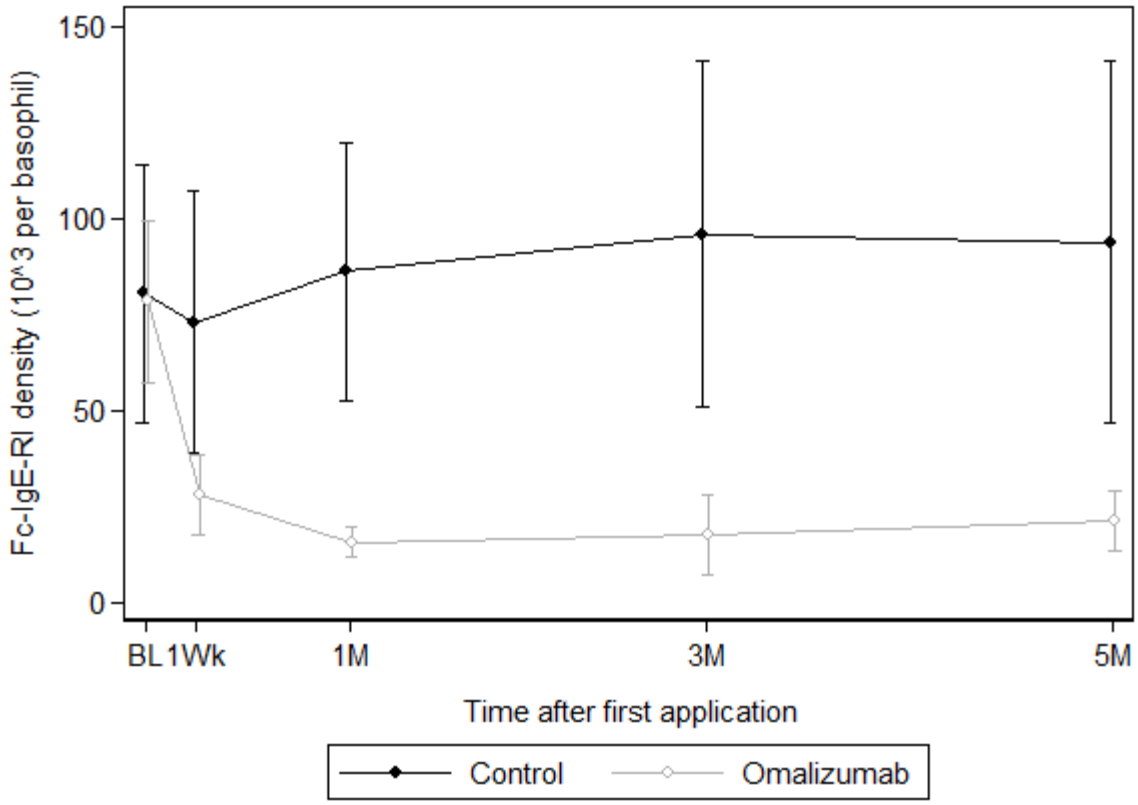

Figure 2 FcεRI receptor density (mean and 95% CI) at baseline, 1 week, 1 month, 3 months, and 5 months (crude values)

Table 2a Basophil releasability after stimulation with anti-IgE (not omalizumab)

Variable	**Visit no.**	**Placebo (median [IQR])**	**Omalizumab (median [IQR])**	**p (unadj.)**	**Ratio adjusted for baseline (95% CI)**	**p (Ratio)**
Basophil releasability (LC50) without IL-3 (ng/ml)	Baseline	29.50 (69.61)	27.95 (57.80)	0.903	0.68 (0.25, 1.83)*	0.446*
	1 Week	39.95 (103.75)	57.20 (228.64)		0.77 (0.20, 3.00)	0.691
	3 Months	93.60 (86.20)	23.30 (31.86)		0.33 (0.07, 1.52)	0.142
Basophil releasability (LC50) with IL-3 (ng/ml)	Baseline	12.55 (19.27)	8.27 (12.85)	0.903	2.03 (0.85, 4.81)*	0.110*
	1 Week	12.23 (38.91)	32.00 (49.26)		1.24 (0.40, 3.86)	0.693
	3 Months	20.70 (58.03)	30.65 (143.80)		2.81 (0.62, 12.71)	0.164

Values are calculated as LC50 (Log10 of anti-IgE concentration in ng/ml resulting in half-maximal activation of basophils). Higher values indicate lower basophil releasability. Median and interquartile range (IQR) are each measured with two methods. P (unadj.) from Wilcoxon's rank-sum test for baseline values. Ratios and p for ratios calculated from linear regression models on log-transformed data for specific times, or from a mixed-effects model including patient ID as a random effect for a repeated measures analysis (*).

Table 2b Incubation of patient serum with donor basophils (CU-BAT).

Variable	Visit no.	Placebo (median [IQR])	Omalizumab (median [IQR])	p (unadj.)	Ratio adjusted for baseline (95% CI)	p (Ratio)
CU BAT: CD63 (%)	Baseline	12.05 (10.80)	18.35 (17.77)	0.244		
	3 Months	10.75 (7.35)	8.35 (15.20)		0.90 (0.42, 1.92)	0.778
CU BAT: CD63 (Stimulation index)	Baseline	4.20 (5.50)	5.00 (7.20)	0.535		
	3 Months	3.20 (2.45)	2.75 (3.50)		0.93 (0.44, 1.99)	0.847

CD63 served as an activation marker and is expressed as the percentage of activated basophils. The stimulation index is expressed as the percentage of activated basophils after stimulation with patient serum divided by the percentage of activated basophils without stimulation (negative control). Median and interquartile range (IQR) are each measured with two methods. P (unadj.) from Wilcoxon's rank-sum test for baseline values. Ratios and p for ratios calculated from linear regression models on log-transformed data for specific times, or from a mixed-effects model including patient ID as a random effect for a repeated measures analysis (*).

Table 3 Urticaria activity score over 1 week (UAS7) and itch severity score (ISS)

Measure	Placebo (n=8)	Omalizumab (n=17)	OR (95% CI)
Baseline			
UAS7 ≤ 6	2	6*	1.47 (0.26, 8.43)
UAS7 = 0	0	3*	4.10 (0.19, 89.44)
ISS = 0	1	3*	1.21 (0.15, 9.92)
3 Months			
UAS7 ≤ 6	2	13	7.80 (1.28, 47.53)
UAS7 = 0	0	8	15.21 (0.76, 305.06)
ISS = 0	0	8	15.21 (0.76, 305.06)
5 Months			
UAS7 ≤ 6	2	9	2.91 (0.52, 16.35)
UAS7 = 0	1	4	1.67 (0.21, 12.97)
ISS = 0	1	4	1.67 (0.21, 12.97)

Confidence intervals from penalized logistic regressions.

Urticaria activity score (UAS7) was summed for a week before the above times and evaluated for the proportions of patients with mild (UAS7≤6) or no symptoms (UAS7=0). ISS was evaluated for the proportion of patients with no itching (ISS=0) in the week before the corresponding visit. Odds ratios (OR) for the UAS7 and ISS were calculated.

* At time of study inclusion, all patients had UAS7>6.

Table 4

Comparison of FcεRI density (receptors per basophil), incubation of patient serum on donor basophils (CU-BAT: CD63 %), and total IgE (kU/L) in partial/non-responders, slow responders, and early responders

	All	Placebo	Partial/Non-responders	Slow responders	Early responders	p Value
	n (%) or median (IQ-range)	n (%) or median (IQ-range)	n (%) or median (IQ-range)	n (%) or median (IQ-range)	n (%) or median (IQ-range)	
Total N	N=25	N=8	N=8	N=2	N=7	
FceRI density at baseline	90.9 (55.9; 138.1)	97.6 (46.8; 133.9)	55.9 (28.6; 87.9)	113.7 (71.2; 156.2)	104.0 (81.0; 162.8)	0.094
Difference of FcεRI density (Basline to 3 months)	-48.0 (-89.1; 5.8)	7.8 (-15.0; 16.9)	-45.3 (-74.9; 0.3)	-99.7 (-138.9; -60.5)	-91.7 (-154.3; -63.7)	0.002
CU-BAT at baseline (%) (BL)	13.8 (7.8; 27.8)	11.6 (7.2; 17.9)	26.3 (8.7; 44.6)	20.3 (13.7; 26.9)	17.1 (4.5; 24.8)	0.438
Difference of CU-BAT (Baseline to 3 months)	-3.2 (-14.5; 0.4)	0.2 (-9.7; 2.1)	-4.6 (-25.9; 2.2)	-17.0 (-24.1; -9.8)	-4.9 (-13.1; 0.1)	0.408
Total IgE at baseline (kU/L)	137.0 (37.8; 311.0)	222.0 (55.8; 1377.8)	25.0 (10.8; 226.5)	296.0 (273.0; 319.0)	113.5 (59.5; 368.5)	0.182

We compared different biological effects among the patients classified into 4 groups (placebo, partial/non-responder, slow responder, and early responder) using the chi-squared test and the non-parametric Kruskal-Wallis rank test as appropriate. We compared the FcεRI receptor density, CU-BAT results (i.e., CD63 change), and total IgE values at baseline, and calculated whether there was a difference of FcεRI receptor density and CU-BAT between baseline and 3 months.

Supplementary material

Table 5 Summary of adverse events by body system (count per group)

Body system	Intervention		
	Placebo	Omalizumab	Total
	No.	No.	No.
Cardiovascular disorder	2	1	3
General disorder	6	6	12
Infections	3	3	6
Musculoskeletal and connective tissue disorder	0	1	1
Nervous system disorder	1	1	2
Skin tissue disorder	0	1	1
Total	12	13	25

Table 6 FcεRI receptors per basophil: estimates and summaries

Analysis	Placebo group	N	Omalizumab group	N	Ratio adjusted for baseline (95% CI) ITT	p	PP	p
Overall FcεRI density		10		20	0.32 (0.23 ,0.44)	<0.001	0.22 (0.14, 0.33)	<0.001
FcεRI density at each visit								
Baseline	80.31 ± 47.18x10^3	10	78.29 ± 45.09x10^3	20	0.97 (0.63, 1.50)	0.908	0.82 (0.56, 1.19)	0.288
1 Week	72.89 ± 47.79x10^3	10	27.83 ± 20.87x10^3	18	0.48 (0.31, 0.73)	0.001	0.37 (0.25, 0.54)	<0.001
1 Month	86.09 ± 43.60x10^3	9	15.46 ± 8.41x10^3	20	0.23 (0.15, 0.34)	<0.001	0.16 (0.11, 0.24)	<0.001
3 Months	95.88 ± 54.17x10^3	8	17.47 ± 21.41x10^3	18	0.20 (0.12, 0.35)	<0.001	0.13 (0.09, 0.17)	<0.001
5 Months	93.81 ± 56.50x10^3	8	21.09 ± 15.23x10^3	17	0.36 (0.19, 0.68)	0.002	0.23 (0.15, 0.35)	<0.001

Ratios of omalizumab over placebo taken from the generalized estimation equations (GEE) model with log link and negative-binomial distribution due to highly skewed distributions and heteroscedasticity. For intention-to-treat (ITT) analysis, all patients were included in the groups they were randomized to; missing values were imputed using multiple imputations. Summary statistics (mean ± SD) are given for times at and after the first application of omalizumab; GEE ratios are adjusted for baseline values. PP = Per protocol.

Table 7 – Summary of rescue medication usage during the whole trial period

Medication	Cumulative frequency (95% CI)		Percentage of patients taking rescue medication		Cumulative frequency. per day (95% CI)	
	Placebo	Omalizumab	Placebo	Omalizumab	Placebo	Omalizumab
H1 Anti-histamines	382.0 (255.1, 508.9)	347.0 (239.7, 454.3)	100.0 (69.2, 100.0)	100.0 (83.2, 100.0)	2.8 (2.1, 3.5)	2.5 (1.8, 3.3)
H2 Anti-histamines			40.0 (12.2, 73.8)	30.0 (11.9, 54.3)		
Prednisone			0.0 (0.0, 30.8)	10.0 (1.2, 31.7)		
Montelukast	77.8 (20.5, 135.1)	54.9 (26.5, 83.2)	60.0 (26.2, 87.8)	65.0 (40.8, 84.6)	1.0 (1.0, 1.0)	1.0 (0.9, 1.0)

The percentages of patients who used rescue medications and the frequency of use of the rescue medications are summarized by means of descriptive statistical techniques (per group).