Treatment optimisation in chronic urticaria and angioedema

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Treatment optimisation in chronic urticaria and angioedema

Optimalisatie van de behandeling van chronisch spontane urticaria en angio-oedeem

(met een samenvatting in het Nederlands)

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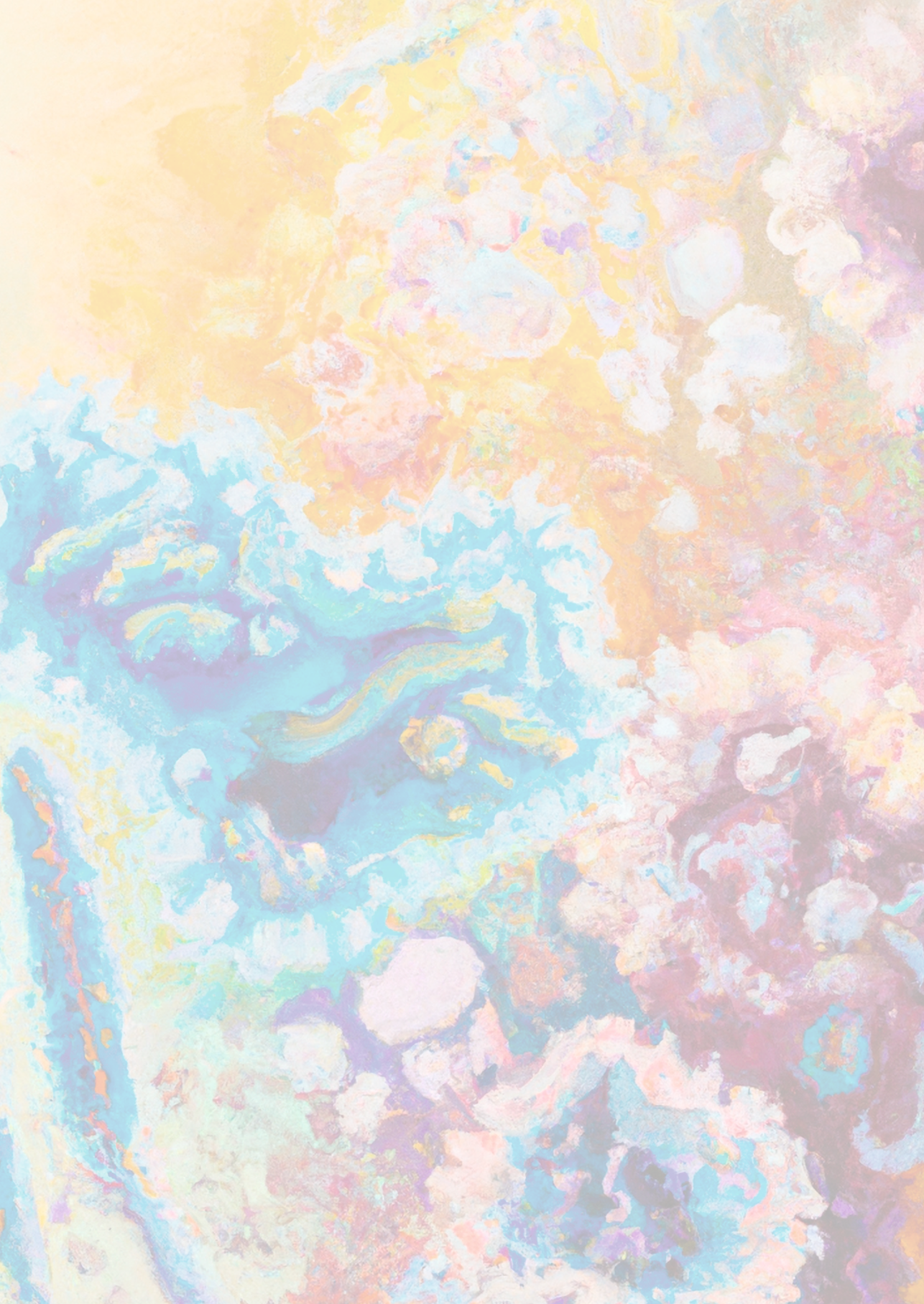

CHAPTER 1

General introduction

CHRONIC SPONTANEOUS URTICARIA AND ANGIOEDEMA, A SINGLE CONDITION OR MULTIPLE SEPARATE IDENTITIES?

Nomenclature and classification of urticaria and angioedema

Urticaria is a skin disorder characterised by pruritic wheals, which usually last for 30 minutes up to 24 hours.[1] In approximately 50% of cases, chronic spontaneous urticaria (CSU) also presents with Angioedema (AE), which is characterized by swelling of the subcutis and lower dermis or mucous membranes. AE can also occur as separate entity either or not combined with wheals, that occur occasionally. Both are considered a spectrum and therefore generally covered by the term CSU. AE can be life threatening when swelling of tong, oral mucosa or larynx is obstructing the airway. While single urticaria lesions are volatile, an AE attack can last for several days.

Urticaria is defined as chronic if the disease lasts six weeks or longer. Chronic urticaria (CU) can be divided into chronic spontaneous urticaria (CSU) and chronic inducible urticaria (CindU). As the name indicates, is the latter dependent on external triggers, and can be differentiated depending on the type of physical trigger e.g. symptomatic dermographism (urticaria factitia), cold urticaria, delayed pressure urticaria, solar urticaria, cholinergic urticaria, heat contact urticaria, aquagenic urticaria, contact urticaria and vibratory urticaria. CSU and CindU can also occur simultaneously.

The reported lifetime prevalence of CSU ranges, but has been estimated to be around 1.4%, with individuals aged 20 to 40 being the most affected.[2] Estimated spontaneous remission of CSU occurs in 17% at year 1, 45% at year 5 and 73% at year 20.[3] Patients with CSU symptoms experience a strong reduction in quality of life that is mainly due to pruritus, sleep problems and aesthetics. This negatively affects emotional well-being and work performance.[4, 5]

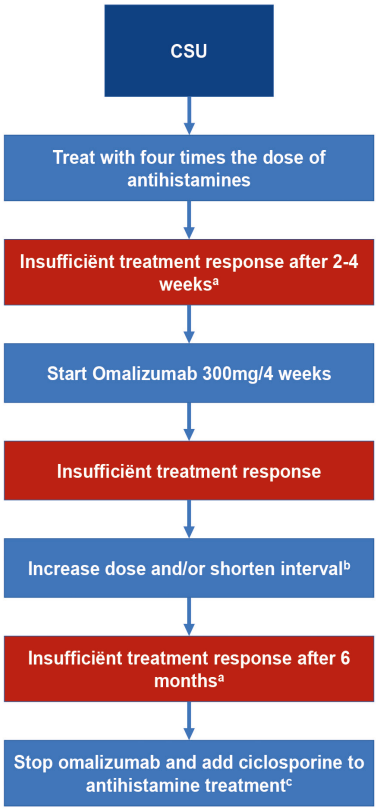

Figure 1. Current international step based model for the treatment of chronic spontaneous urticaria. Adapted from Zuberbier et al 20226

Legend: CSU; Chronic Spontaneous Urticaria. Additionally; Short treatment courses with glucorticosteroids could be considered at any time during the step based model in severe cases.

[a]Or earlier in case of intolerable symptoms

[b]up to 600mg every 2 weeks[c]Up to 5mg/kg body weight

Diagnostics and treatment of CSU

CU is diagnosed based on the characteristical clinical features and careful patient history including potential underlying triggers as allergies and infection. Possible laboratory tests include leucocyte differentiation, CRP and/or BSE, total IgE and IgG anti-TPO (and in case of AE; C4) in order to rule out underlying allergy, infection or inflammatory disease. To guide and evaluate treatment decisions, the continuous use of validated patient related outcome measurements (PROMS) is recommended, including the Urticaria Control Test (UCT), Urticaria Activity Score summed over 7 days (UAS7) and the Angioedema Activity Score (AAS).[6] Following international guidelines, CU is treated according to a stepped-based model starting with a single dose of second-generation H1-receptor antagonists (Figure 1).[6] If a single dose of antihistamine is insufficient, it is recommended to increase the dose to fourfold the standard dose.

Oral corticosteroids can be used for exacerbations, but should be limited to short-term treatment. Approximately 45% of patients do not respond well to a fourfold dose of antihistamines.[7] These patients are eligible for treatment with omalizumab, starting with a dose of 300mg every 4 weeks.

Omalizumab has been demonstrated as an effective and safe option in the treatment of CSU, with initial clinical trial results reporting good or complete response (UAS7<6) in 52% of patients.[8] Response rated in real world studies were later found to be even higher, with a recent review reporting treatment effectiveness in 85% of patients of which 57% report complete response.[9] Still, insufficient response is seen 15% of patients treated with standard doses of omalizumab.[10, 11] In those cases it is recommended to increase the treatment dose or to shorten treatment intervals. Due to lack of evidence in the literature, this is mainly based on expert opinion. Only three small studies investigated the benefit of updosing omalizumab in patients with insufficient disease control on standard dose and reported therapy response (complete or partial response) to higher doses of omalizumab ranging from 45% to 83%.[12-14]

Long term perspective in omalizumab treatment

In patients who do experience prolonged complete treatment response, treatment could be tapered off and if successful discontinued. The ideal strategy of dose reduction or manner of discontinuation however, remains unknown and is not addressed in international guidelines.[6] In the Dutch guideline it is recommended to taper off omalizumab treatment interval when (almost) complete remission is achieved, by progressively increasing the dosing interval every visit by one week, up to eight weeks after which treatment can be discontinued.[15] However this is based on expert opinion and has to be supported by adequate studies.

So far, only a few retrospective studies with small patient numbers regarding omalizumab discontinuation and minimal interval extension have been performed.[12, 14, 16-18] These studies successfully extended the interval between administrations by one week per administration in patients who maintained well-controlled disease from five to twelve weeks, suggesting that extending the interval between administrations in omalizumab treatment could be possible while retaining effectiveness. In some patients this might be due to spontaneous remission, while in others this is possible despite 'active' disease, since stopping, or extending the interval too much results in exacerbation.

The performance of omalizumab in a paediatric and adult population using drug survival analysis.

Generally, the primary efficacy and safety of a new drug is investigated in randomized controlled trials (RCT) measuring therapy response and side effects in a protocolized manner, mostly with short follow-up. RCTs are characterized by highly regulated treatment protocols, and specifically selected patients. In daily practice however,

drugs are used over extended periods of time, sometimes in patients with several comorbidities, and often in combination with other drugs. Furthermore, patients and doctors preferences, reimbursement and guidelines impact treatment decisions in daily practice substantially. Therefore the performance of a drug in terms of effectiveness and safety differs from that in RCTs. Drug survival analysis is a method to measure the expected period of time until a certain event, in this case discontinuation of the drug for a specific defined reason (e.g. disease control, side effects, ineffectiveness or other). This makes it one of the most comprehensive methods to evaluate a drugs' performance in daily practice. By analysing when and why patients discontinue treatment and which factors promote or delay treatment discontinuation, a comprehensive estimation of a drugs' performance can be made.[19, 20] Our group was previously the first one to report overall omalizumab drug survival rates in an adult CU population, with 77%, 61% and 55% after 1,2 and 3 years respectively. The favourably properties of omalizumab was demonstrated in daily practice as we found that the majority of patients (49%) discontinued treatment due to well controlled disease. Only 16% discontinued treatment because of ineffectiveness, 11% due to side effects and 7% due to a combination of both. Independent determinants of drug survival related to ineffectiveness and side-effects could not be calculated due to the small sample size. To date, two other centres have performed omalizumab drug survival studies in adult patients with CU.[21, 22]

Ghazanfar et al. (n=154) reported a omalizumab survival rate of 78% after six months in adult patients with CU and Ke et al. reported drug survival rates (n=298) of around 72% for the first year and 60% after 18 months. Both studies were relatively short and did not analyse of reasons of discontinuation. Likewise, no determinants for drug survival were reported.

The role of the complement system and basophils in the aetiology of CSU, and their response to treatment

Skin mast cells are considered the main effector cells in CU as degranulation leads to histamine release and causes increased vascular permeability leading to tissue swelling and stimulation of sensory nerves, which in turn leads to swelling, redness and itching.[23, 24] Mast cells can be activated by crosslinking of IgE through the high-affinity IgE receptor (FcεRI) resulting in activation of the internal signalling pathways syk and lyn.[1] The half-life of serum IgE is only several weeks, that of mast cell-bound IgE is several months. Although the exact triggers of mast cell activation in CU are not known, multiple pathways are thought to play a role in CU. Mast cells can be activated through many membrane receptors, including receptors for complement molecules C3a and C5a, neuroreceptors and codeine receptors. Mast cell activation through the coagulation system has been proposed by Yanase et al. in which activated coagulation factors may induce plasma extravasation and activation of skin mast cells.[25] Upon activation of mast cells, besides histamine, other mediators including proteoglycans, cytokines, proteases, leukotrienes, TNF-α are released.[26] Other cell types thought to also play a role which are found in cellular infiltrates include basophils, monocytes, peripheral blood dendritic

cells, neutrophils, and T-lymphocytes.[27] There is increasing evidence that basophils in particular might play a role in the pathogenesis of CU.[28, 29] Similar to mast cells, basophils are a major source of histamine and expression of the FcεRI. They differ from mast cells as basophils are blood borne cells and circulate in the bloodstream for about 7 days. Furthermore, basophils share a common precursor with eosinophils, while mast cells are more closely related to monocytes.[30] Patients with CU show a decrease in basophil numbers with CU with lower numbers indicating a more severe disease.[31] Basophils have found to be present in lesional skin of CSU patients indicating migration from the blood to the skin.[31, 32] Since the working mechanism of omalizumab is based on the binding of free circulating IgE, leading to down-regulation of FcεRI on mast cells, it is hypothesized that also other FcεRI bearing cells as basophil cells in blood are involved.[29, 33] FcεRI expression on basophils might play a role in omalizumab effectiveness and offer a possible tool for predicting treatment response.[34, 35] We therefore investigated the role of basophils and other FcεRI bearing leucocytes in patients with CSU and it's relation to omalizumab treatment.

Complement C5a as a specific activator of mast cells and basophils in CSU.

The complement system is known for its rapid acting pathway, which fits with the clinical properties of urticaria, with wheals occurring within hours, and may therefore be involved in its pathogenesis. Components of the complement system can degranulate various leukocytes through their designated receptors found on the cells surface on mast cells, basophils, eosinophils, neutrophils and monocytes.[36, 37] In the classical pathway, C1 is activated through immunoglobulin binding, whereas the alternative cascade starts by cleaving C3 into C3b and C3a. C5 in turn is cleaved by C3b into C5b and C5a.[38] An increase in C3 and C4 levels has previously been found in a small population of CU patients with severe disease, but was not found in mild to moderate disease. The role of C5a in particular has been studied in CU, as it can activate mast cells and basophils through the C5a receptor.[38] The C5a receptor was found to be exclusively expressed on the surface of skin mast cells, and not on other human mast cells. One study suggested an increased plasma level of C5a in patients with CSU, but did not correlate this increase to clinical response to treatment.[39] The exact role of C5a and its clinical relevance in pathogenesis of CU is therefore unknown.

Treatment strategies and their limitations in idiopathic angioedema without wheals
The characteristic swellings of AE are suggested to be mediated by two vasoactive peptides; namely, histamine or bradykinin. AE with wheals is generally considered part of CSU and thought to be histamine mediated. AE without wheals is classified as hereditary (HAE) or acquired (AAE).[40] While HAE is bradykinin mediated, AAE without a known cause is currently differentiated based on its clinical response to treatment into idiopathic histaminergic AAE (IH-AAE) and idiopathic non-histaminergic AAE (Inh-AAE), with the latter not responding to antihistamine therapy.[41] In hereditary angioedema (HAE) an excess of plasma levels of bradykinin is formed due to C1-esterase inhibitor (C1-INH) deficiency. Patients with HAE are treated by supplementing C1-INH. The pathomechanism of attacks in InH-AAE remain unknown. Due to international CSU guidelines, patients with InH-AAE, after excluding acquired C1-INH deficiency, are currently considered as suffering from (a subtype of) CSU and treatment with omalizumab is advised.[42] Effectiveness of omalizumab in AO without wheals is so far demonstrated in only seven small case series reporting positive results.[43, 44] However, such studies are subject to publication bias.

The limited available studies suggest that omalizumab is effective in only part of the patients suffering from Inh-AAE. In Inh-AAE patients with insufficient omalizumab response no alternative treatment options are currently approved. A variety of prophylactic therapeutic options are available for patients with proven bradykinin mediated AE (HAE), including plasma derived C1-INH (pdC1-INH), recombinant human C1-INH (rhC1-INH), ecallantide and tranexamic acid. The effectiveness of plasma derived C1-INH in patients with Inh-AAE is described in three case reports with only four patients.[45-47] The idea to introduce these type of treatment in patients with InH-AAE is supported by studies showing elevated bradykinin levels in four patients with InH-AAE during acute attacks compared to normal levels during remission and in healthy controls.[48] We hypothesize, that InH-AAE is at least in part bradykinin mediated and some patients could benefit from bradykinin targeted therapy.

OUTLINE OF THIS THESIS

In this thesis we aim to optimise treatment for patients with chronic urticaria and idiopathic angioedema with and without wheals. We investigated clinical patterns in search for a more personalised treatment approach, as well as tools for predicting treatment effectiveness.

The objective of the first two studies is to determine the performance of omalizumab and possible predictors and determinants of drug survival in daily practice in adults **(chapter 2)** and children **(chapter 3)** with CSU**.** We investigated the effect of updosing omalizumab (**chapter 4)** and treatment interval prolongation **(chapter 5)** in order to realize a more personalised treatment approach and achieve optimal effectiveness for patients with

severe CSU. To obtain a more comprehensive impression of the differences within patients with idiopathic angioedema (with or without wheals), we focussed on profiling patients with idiopathic angioedema based on attack frequency and disease control in relation to treatment response **(chapter 6).** In order to gain a better understanding of the pathomechanism of CSU and the working mechanism of omalizumab, we investigated the role of the complement system **(chapter 7)** and FcεRI-bearing leucocytes **(chapter 8)** in relation to clinical treatment response. In **(chaper 9)** we present the results of a prospective pilot study investigating the effectiveness of recombinant human C1-INH in 6 patients with InH-AAE in relation to biomarkers of the bradykinin pathway.

The implications of our findings are discussed and further perspectives considered in **chapter 10**.

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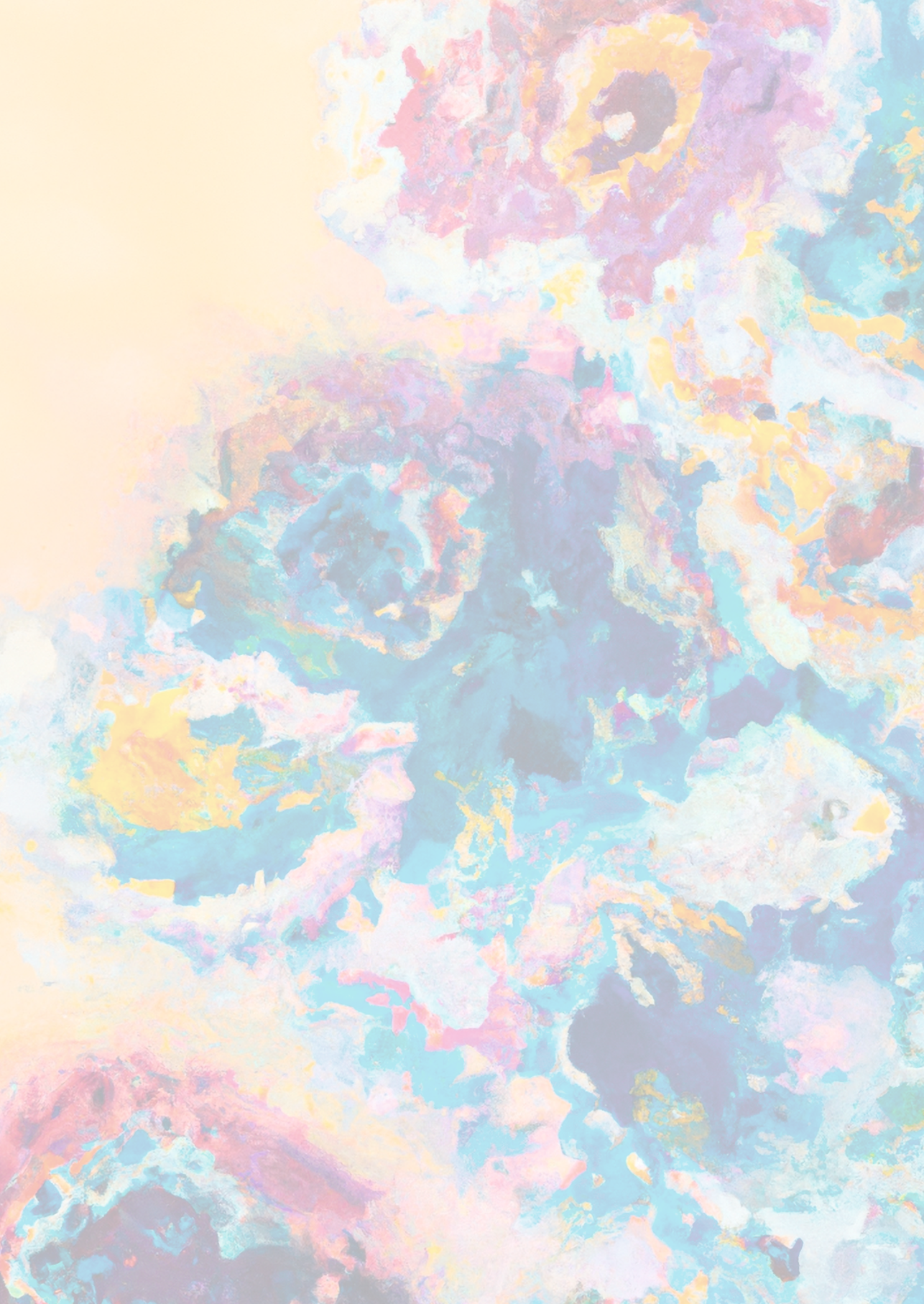

PART I

Optimization of omalizumab treatment in adult and paediatric patients with chronic urticaria

CHAPTER 2

Timing of response and disease duration before start are important determinants of omalizumab drug survival in chronic urticaria

Manuscript in preparation

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ABSTRACT

Background: Omalizumab is used for the treatment of chronic urticaria (CU) and appeared safe and effective in numerous randomized controlled trials (RCTs). Large daily practice studies are limited and often of short duration or focus only on effectiveness or side effects.

Objective: In this large multi-centre prospective daily practice cohort study we used drug survival analysis to assess the performance of omalizumab in patients with CU over a period of up to ten years.

Methods: Data of all patients with CU treated with omalizumab in two Dutch centres was extracted from the electronic patient records. Drug survival was analysed by Kaplan–Meier survival curves, determinants of drug survival were analysed using univariate and multivariate Cox-regression analysis with backward selection. Side effects since the start of treatment were described in detail.

Results: 659 patients (72 % female; mean age 42, 1162 treated patient-years) with a omalizumab follow-up time up to 10 years were analysed. The overall drug survival rates for omalizumab decreased from 76% to 37%, after one year to 5 years, respectively. Of the 276 patients who discontinued treatment, 172 (62%) discontinued treatment due to well-controlled disease and 39 (14%) due to ineffectiveness and 22 (8%) due to side effects. Fast response resulted in a higher chance to discontinue treatment due to well-controlled disease and a lower chance to discontinue treatment due to side effects. A shorter disease duration before starting omalizumab let to a higher chance to discontinue treatment due to well-controlled disease and a lower chance to discontinue treatment due to side effects. The use of immunosuppressive drugs at the start of treatment was associated with a higher chance of discontinuation due to ineffectiveness.

Conclusion:
Omalizumab demonstrated to be effective and safe in a large daily practice study, with well-controlled disease as the main reason of treatment discontinuation..

The identified determinants of drug survival provide possibilities for anticipating treatment duration and managing patients' and doctors' expectations.

INTRODUCTION

Chronic urticaria (CU) is characterized by severely itching wheals, for at least 6 weeks, with or without angioedema (AE) and is differentiated between Chronic Spontaneous Urticaria (CSU) and Chronic Inducible Urticaria (CindU).[6] Patients with urticaria have a strongly reduced quality of life.[49] In randomized controlled trials (RCTs) Omalizumab was proven a safe and effective treatment option for patients with CSU in case of insufficient response to a fourfold dose of antihistamines.[50] Daily practice data of omalizumab are limited investigating population with short follow-up period or focus only on effectiveness or side-effects.[51] Drug survival analysis is a method to measure a drugs' performance in daily practice, which incorporates both effectiveness and safety, as well as other less measurable factors including preferences of both patients and doctors.[19, 20] A drugs survival curve is often visualised with Kaplan-Meier curves and such DS analyses are frequently used in chronic diseases that require long-term treatment including psoriasis and atopic dermatitis.[19, 52] It measures the expected period of time until a certain event, in this case discontinuation of the drug, visualised by a step down in the Kaplan–Meier curve at that time point. Drug survival uses the technique of censoring (visualized by a tick mark in the curve) to incorporate patients' data from the start of treatment up to the end of follow-up. To date, three omalizumab drug survival studies have been performed in two adult[22, 53] and one paediatric population,[54] respectively. Ke et al. reported drug survival rates of adult patients with CU (n=298) of around 72% for the first year and 60% after 18 months, but did not analyse reasons of discontinuation and determinants for drug survival. Our group previously reported overall drug survival rates in an adult population of 77%, 61% and 55% after 1,2 and 3 years respectively.[53] Well-controlled disease was the main reason of treatment discontinuation (49%), followed by ineffectiveness (16%), to side effects (11%) or a combination of both side effects and ineffectiveness (7%). CindU was identified as a determinant for an overall longer drug survival and a longer drug survival related to well-controlled disease activity. Independent determinants related to ineffectiveness and side-effects could not be calculated due to the small sample size. Our group also reported 1-year and 2-year drug survival rates of 62% and 50% in a paediatric population. Well-controlled disease was the most frequent reason of discontinuation (24%), followed by ineffectiveness (8%) and side effects (3%). Reasons of discontinuation and determinants for drug survival were likewise not determined due to the small sample size. In the present dual-centre daily practice cohort study we aimed to show the performance of omalizumab for adult patients with CU with the most comprehensive omalizumab drug survival analysis to date. This allowed for differentiation between reasons of discontinuation and determinants for omalizumab drug survival related to well-controlled disease, ineffectiveness and side effects.

METHODS

Patient population

In this multicentre study all adult patients with chronic urticaria (wheals, AE or CindU) who were treated with omalizumab in the department of Dermatology of Erasmus Medical Center Rotterdam from October 2014 till September 2021 and the Dermatology/ Allergology of University Medical Centre Utrecht from February 2012 till May 2021 were included. Patient data were retrospectively obtained from the Electronic Medical Records. Patients were excluded when omalizumab was prescribed for a diagnosis other than CU.

The main CU symptom diagnosis was differentiated into three groups: CSU with mainly symptoms of wheals (CSU-wheals), CSU with mainly angioedema (CSU-AE) and CindU, based on medical history taking and physical examination. When a patient had more than one component of CU, for example both wheals and AE, the subordinate symptom was collected as a CU-comorbidity. The following variables were collected; age, gender, presence of autoimmune disease, main symptom, CU comorbidity, time of onset of CU, use of immunosuppressive drugs at the start and during omalizumab treatment, time to start self-administration, the time between stop and restart of omalizumab, the occurrence of side-effects, use of antihistamines, and other drugs, treatment dosage of omalizumab, number of administrations and reasons for discontinuation. Disease-activity scores, Urticaria Control Test (UCT), Urticaria Activity Score summed over seven days (UAS7), and the Angioedema Activity Score (AAS) were collected at baseline, at the second administration (T2), at the fourth administration (T4), and at the end of every treatment episode or data lock (T-end). Data regarding part of the population (n=142) has been published before by Spekhorst et al.[53]

Treatment guidelines

Patients were treated according to the local guidelines, which were based on the national and international guidelines, and largely comparable in both centres. Briefly, treatment with omalizumab is initiated in patients with high disease activity scores (UCT ≤11 and UAS7 ≥ 16) despite the use of antihistamines in fourfold the licensed dose. Initial treatment consists of 300 mg omalizumab every four weeks. After six administrations (UMCU) or three administrations (EMC) of omalizumab, treatment response is evaluated. In case of insufficient response, the dose is increased to 450 mg or 600 mg. When treatment was effective, the time interval between administrations is incrementally extended to eight weeks. Omalizumab is discontinued if the time interval could successfully be extended to eight weeks. If symptoms reoccurred after discontinuation despite fourfold antihistamine treatment, a restart of omalizumab treatment is indicated. In the Netherlands, omalizumab is reimbursed for CSU for an indefinite time. For the treatment of CindU, written permission must be requested from the patients' health insurance every six to 24 months.

Disease outcomes

CU disease activity was measured using UAS7, AAS, and UCT questionnaires. Patients were classified as complete, good, partial or poor responders. When the UAS7-score was equal to zero or UCT was equal to sixteen, patients were classified as a complete responder. Good response was defined as a UAS7 ≤6 or UCT ≥12.[55] If a UAS7 ≤6 or UCT ≥12 was not reached, a decrease in the UAS7-score of 10 points or more[56] or an increase in UCT-score of 3 or more[57] (minimal important difference, MID) was considered a partial response. Patients who did not met these criteria were defined as poor responders. The onset of response to omalizumab was differentiated as fast response (UAS7-score ≤ 6 or the UCT-score **≥** 12 within 4 weeks after the first administration (at T2)) and 'delayed response' (UAS7-score ≤ 6 or the UCT-score ≥ 12 after the first administration (T4 or Tend)). Missing disease activity scores were supplemented by analysing the (onset of) response based on the physicians' description in the electronic medical records or if available, disease activity scores with a maximum of 4 weeks away from original measure point were used.

Drug survival

Drug survival was determined through Kaplan-Meier survival curves. Overall, four drug survival events were defined and analysed separately: any discontinuation (overall drug survival) (I), discontinuation due to well-controlled disease activity (II), discontinuation due to side effects (III), discontinuation due to ineffectiveness (IV). When patients discontinued due to both ineffectiveness and side-effects, they were considered to have an event in both sub analyses (III and IV). Patients were censored when still using omalizumab at time of data lock, or when lost to follow up. When patients discontinued for other reasons, they were considered to have an event in the overall drug survival analysis (I) but were censored in the sub analyses (II, III and IV).

If patients started omalizumab in another centre, the start date in the original centre was used. When omalizumab treatment was discontinued for more than 90 days and was restarted, this subsequent treatment course was considered as a second treatment episode.

Statistical analyses

Graphs were made using GraphPad Prism 8.3. Normally distributed data were presented as mean with standard deviation (SD), when data were not normally distributed, data were presented as median with interquartile range (IQR). Categorical variables were presented as numbers with percentages. Comparisons among groups were made by unpaired t-test or Mann-Whitney U test for continuous variables that were normally or non-normally distributed respectively, whereas categorical variables were compared using the chi-squared test. Statistical significance was assumed for P-values ≤0.05. Determinants of drug survival were further selected by comparing patients on each potential predictor in a univariate Cox regression model. Determinants that differed between the two groups with a P-value ≤0.2 were entered in a multivariate Cox

regression model. With backward selection, a full model was built. Missing data was excluded from the analysis. Statistical analyses were performed in SPSS IBM SPSS Statistics 26 (IBM, Armonk, NY, U.S.A.).

This study was approved by the Medical Ethical Review Committee (18/862).

RESULTS

A total of 659 patients (mean age 42 ; 72 % female), who started treatment between February 2012 and November 2021 (UMCU: 5th of May 2021; EMC 15th November 2021), were included, of which 302 patients were treated in the EMC and 357 in the UMCU (Table 1). Treatment data comprised a total of 1162 patient-years on omalizumab (including drug interruptions < 90 days). The maximum treatment duration was 7.9 years.

The majority of patients (85%) suffered mainly from wheals, whereas mainly AE or mainly CindU symptoms were both reported in 7% of patients (Table 1). Patients who mainly suffered from wheals, reported concomitant AE in 32% of cases, CindU in 32% and a combination of both AE and CindU in 20% of cases. In 324 patients (49%) CindU was diagnosed as main or concomitant symptom. Pressure urticaria (n=167, 52%) represented the largest group of CindU subtype, followed by urticaria factitia (n=111, 34%). CindU subtypes with a lower frequency were cholinergic urticaria (n=91, 28%), cold urticaria (n=32, 10%), heat contact urticaria (n=16, 5%) and urticaria solaris (n=11, 3%).

Nearly all patients (n=625, 95%) used antihistamines throughout omalizumab treatment, of which 66% (n=437) at least four times a day: the recommended dose before start of omalizumab (Table 2). Immunosuppressive drugs targeting CU at the start of treatment were used in addition to antihistamines in a relatively large proportion of patients (30%, n=198) and mainly concerned of corticosteroids (25%, n=165).

	Total	UMCU (NL)	EMC (NL)
n	659	357	302
Female	474 (72%)	257 (72%)	217 (72%)
Age at start treatment mean in years (SD; min-max)	42 (16; 12-87)	41 (16; 14-83)	43 (16; 12-87)
< 18 years	42 (3%)	8 (2%)	5 (2%)
Disease duration*			
More than 2 years	338 (51%)	184 (52%)	154 (51%)
More than 5 years	195 (30%)	103 (29%)	92 (31%)
Autoimmune disease	**139 (21%)[a]**	49 (14%)	90 (30%)
Main symptom			
Wheals	563 (85%)	313 (88%)	250 (83%)
AE	49 (7%)	29 (8%)	20 (7%)
CindU	**47 (7%)[b]**	15 (4%)	32 (11%)
CU comorbidity†			
Wheals	**18 (3%)[c]**	17 (5%)	1 (0.3%)
AE	**294 (45%)[d]**	175 (49%)	119 (39%)
CindU	**277 (42%)[e]**	132(37%)	145(48%)
Immunosuppressive concomitant drugs at start of omalizumab	**198 (30%)[f]**	139(39%)	59(20%)
Prednisone	**165 (25%)[g]**	120(34%)	45(15%)
Baseline score (mean, SD)#			
UAS7	**26 (10)[h]**	25 (11)	27 (9)
UCT	**4 (3)[i]**	5 (4)	4 (3)
AAS	39 (35)	34 (27)	49 (47)
Treatment response (n/available scores)			
Fast responders	264/527 (41%)	159/303 (47%)	105/224 (35%)
Responders T4	**304/473 (46%)**	193/280 (54%)	111/193 (37%)
Complete or good Respons at Tend	464/633 (73%)	261/341 (77%)	203/292 (70%)
Partial or Non-respons at Tend	**169/633 (27%)**	80/341 (22%)	89/292 (30%)

Table 1. Baseline patients characteristics *data of 16 patients missing. †: comorbidity subordinate to main symptom ; #: score before start omalizumab treatment, median (IQR 25-75). Significant differences (p< 0.05) between centres are marked bold. Significant differences between UMCU and EMC: [a] p=0.000, [b] p=0.001, [c] p=0.001, [d] p=0.013, [e] p=0.004, [f] p=0.000, [g] p=0.000, [h] p=0.014, [i] p=0.000. Treatment response was based on UAS7 and UCT scores, or if unavailable, the physicians' description in the EMR of complete response. Complete response: UAS7=0, UCT=16 Good response: UAS7 ≤6 or UCT ≥12. Partial response: a decrease in the UAS7-score of 10 points or a rise in UCT score of 3, but not reaching UAS7 ≤6 or UCT ≥12. Poor response: Patients who did meet these criteria.

	Totaal = 659	UMCU = 357	EMC=302
Self administration	380 (58%)	214 (60%)	166 (56%)
High dose omalizumab used[a]	212 (32%)	93 (26%)	119 (39%)
Antihistamine	625 (95%)	350 (98%)	275 (91%)
At least 4dd	437 (66%)	248 (69%)	189 (63%)
Immunosuppressants at start of treatment	198 (30%)	139(39%)	59(20%)
Prednison	165 (25%)	120 (34%)	45 (15%)
Continuous[c]	104 (63%)	66 (55%)	38 (84%)
On demand[d]	61 (37%)	54 (45%)	7 (16%)
Ciclosporin	25 (4%)	16 (5%)	9 (3%)
Immediately stopped	9 (36%)	3 (19%)	6 (67%)
Bridged	11 (44%)	9 (56%)	2 (22%)
Continuous treatment	5 (20%)	4 (25%)	1 (11%)
MTX	4 (1%)	4 (1%)	0 (0%)
Other	18 (3%)	12 (3%)	6 (2%)
Immunosuppressants during treatment[b]	171 (26%)	72 (20%)	99 (33%)
Corticosteroids	79 (12%)	43 (12%)	36 (12%)
Cyclosporine	52 (8%)	15 (4%)	37 (12%)
MTX	15 (2%)	4 (1%)	11 (4%)
Other	25 (4%)	10 (3%)	15 (5%)

Table 2. Medication at start and during omalizumab treatment.

[a]High dose omalizumab: 450mg or 600mg.

[b]Total immunosuppresive drug use at any time during treatment, including rescue medication and drug targeting other diseases. Multiple selections possible.

[c]Continuous use of prednisone surrounding the start of omalizumab treatment

[d]Incidental use of prednisone during a urticaria or angioedema exacerbation surrounding the start of omalizumab treatment

Treatment dose and response.

The maximum treatment dose was 300mg/4 weeks (standard dose) in most of the cases (n=444, 67%), higher doses than the standard dose was used in 32% of patients (n=212). Statistically significantly more patients in the EMC used higher doses (450mg per four weeks or more) of omalizumab compared to patients in the UMCU (39% vs 26% respectively, p<0.001).

Median baseline score for UCT was 4, for UAS7 26, and for AAS 39, indicating active and severe disease in the majority of the patients (Table 1, Supplemental table S1). At the end of treatment or at data lock, 36% of patients (n=239) reported a complete response, 34% (n=225) a good response, 16% (n=108) a partial response and 9% (n=61) a poor response. Good and complete response within the first month (fast response) was achieved in 41% of patients, in 46% of patients after three months and in 70% of patients at the end of treatment or at data lock.

Side effects

In total, 486 patients (71%) cumulatively reported 1235 side effects during treatment (Supplemental table S2). The most frequent reported side-effect was fatigue (17%), followed by headache (13%) and flu-like symptoms (11%). Proportions did not differ between the two centres. One case of suspected anaphylactic reaction was reported, leading to subsequent treatment discontinuation.

Drug survival analysis

At data lock, 51% patients (n=338) were continuously treated with omalizumab. 45% of patients (n=298) made at least one stop attempt of at least 90 days, 37% (n=84) of these patients restarted omalizumab treatment for a second treatment episode. Four percent of patients (n=23) were lost to follow-up (Table 3). The 1, 2, 3, 4, 5 years overall drug survival of omalizumab of the first treatment episode was 75%, 57%, 48%, 42%, and 37% respectively and was mostly determined by well-controlled disease activity (Figure 1; Supplemental table S3). The median overall survival time of omalizumab was 2.6 years. Figure 1 shows the overall drug survival and the drug survival curves differentiated for reason of discontinuation: well-controlled disease activity; side effects and ineffectiveness. The drug survival rates related to well-controlled disease activity were 85%, 69% 61%, 56%, 50% after 1, 2, 3, 4 and 5 years, respectively (Supplemental table S3), approaching overall drug survival rates.

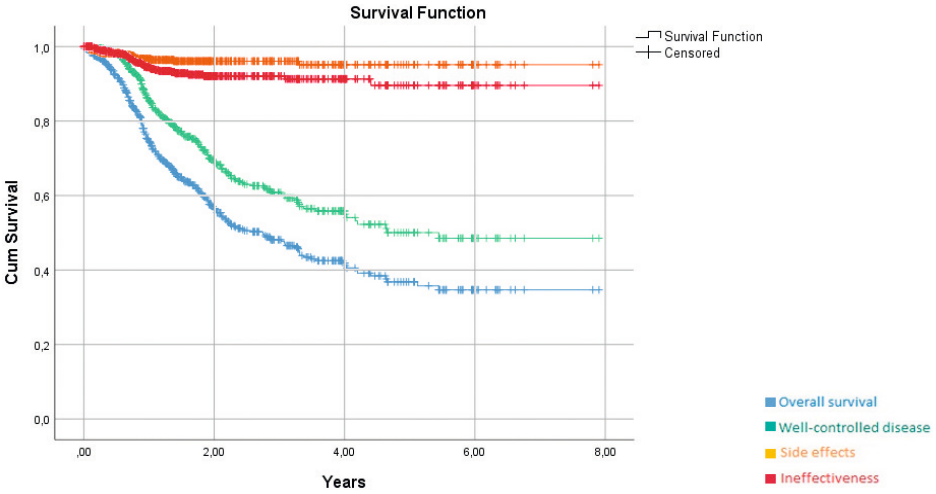

Figure 1. Omalizumab drug survival in Chronic Urticaria
Kaplan-Meier curves representing omalizumab drug survival for the first treatment episode; overall drug survival and differentiated for reasons of discontinuation are shown.

Patients discontinuing treatment	276 (100%)
Reasons of treatment discontinuation	
Remission	172 (62%)
Side-effects	22 (8%)
Ineffectiveness	39 (14%)
Pregnancy wish	19 (7%)
Other[a]	31 (11%)
Unknown	1 (<1%)

Table 2. Reasons of treatment discontinuation (>90 days). 8 patients discontinued treatment due to both side-effects and ineffectiveness, therefore, the separate reasons of treatment discontinuation add up to 284. [a]Reasons of discontinuation including: relocation/migration (9 patients), personal reasons (9 patients), logistic reasons (3 patients), a preference for cyclosporine (2 patients) or various other reasons (8).

The 1, 2, 3, 4 and 5 year drug survival related to discontinuation due to ineffectiveness were 94%, 92%, 92%, 91% and 90% respectively; and related to discontinuation due to side effects 97%, 96%, 96%, 95% and 95% respectively. There was no statistical significant difference in overall drug survival rate, drug survival related to remission, ineffectiveness or side-effects between the two centres.

Reasons for treatment discontinuation

The most frequent reason for discontinuation of omalizumab was well-controlled disease activity (n=172, 62%). Thirty one patients (11%) discontinued treatment solely because of ineffectiveness of which eight were treated for ≤6 months. Nineteen patients discontinued treatment due to wish for pregnancy (7%), 14 patients (5%) solely due to side effects, and 8 patients (3%) due to a combination of both side effects and ineffectiveness. Side-effects as reason for discontinuing omalizumab were tiredness (n=5), headache (n=2), hair loss (n=2), arthralgia (n=2), worsening of disease (n=2), dizziness (n=2), flu-like symptoms (n=1), psychological symptoms (n=1) and a suspected anaphylactic reaction (n=1) (Supplemental table S2) .

Determinants of drug survival

Variables from the univariate analysis, related to well-controlled disease (Supplemental table S4), which were further tested in the multivariate analysis included: Fast response, a disease duration ≥2 years, age at start treatment, wheals as main diagnosis, the presence of CindU. Multivariate analysis showed a disease duration ≥2 years (HR 0.531, 95% CI 0.381-0.738) as an independent determinant of longer drug survival (less likely to discontinue omalizumab), and fast response (HR 1.462, 95% CI 1.047-2.041) and age at start treatment (HR 1.010, 95% 0.999-1.020) for a shorter drug survival (higher risk to discontinue treatment) of omalizumab related to well-controlled disease activity (Figure 2).

Variables from the univariate analysis, related to discontinuation due to ineffectiveness (supplemental table S5), which were further tested in the multivariate analysis included: the presence of CindU and use of immunosuppressive at the start of treatment. Multivariate analysis showed immunosuppressive drug use at start of treatment (HR 1.885, 95% CI 1.003-3.541) for a shorter ineffectiveness-related drug survival.

Variables from the univariate analysis, related to discontinuation due to side effects (supplemental table S6), that were used in the multivariate analysis included: Fast response and a disease duration of ≥2 years. Multivariate analysis showed disease duration ≥2 years (HR 3.443, 95% CI 1.131-10.480) as an independent determinant of shorter drug survival and fast response (HR 0.165, 95% CI 0.048-0.570) of longer side-effect related drug survival.

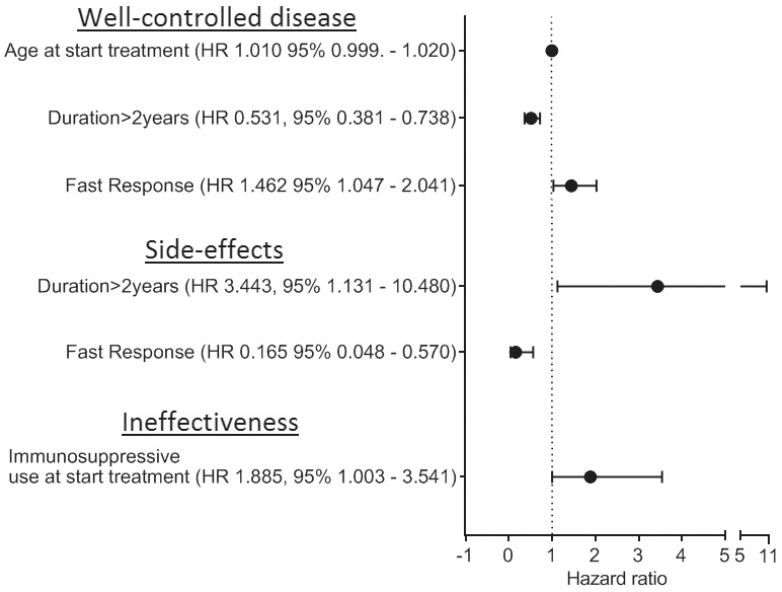

Figure 2. Confidence interval Determinants of omalizumab drug survival in CU per reason for discontinuation resulting from multivariable Cox Regression analysis. Data are presented as hazard ratio (95% confidence interval) for well-controlled disease, ineffectiveness and side effects.

DISCUSSION

This is the first multi-centre study to report determinants of drug survival of omalizumab differentiated for well-controlled disease, ineffectiveness and side-effects in a large population of patients with CU with a long follow-up period of 10 years. The overall drug survival rates were 75%, 57%, 48%, 42%, and 37% after 1, 2, 3, 4, 5 years respectively, and mostly determined by well-controlled disease activity. Sixty-two percent of discontinuations were due to well-controlled disease, infrequent reasons for discontinuing omalizumab were ineffectiveness (11%), pregnancy wish (7%), side effects (5%) or a combination of side effects and ineffectiveness (3%). Fast response (within one month) resulted in a higher chance to discontinue treatment due to well-controlled disease and a lower chance to discontinue treatment due to side effects, and could be considered as a favourable characteristic.

A shorter disease duration before starting omalizumab let to a higher chance to discontinue treatment due to well-controlled disease and a lower chance to discontinue treatment due to side effects. A disease duration of > 2 years could therefore be regarded as a unfavourable characteristic. The use of immunosuppressive drugs at start of omalizumab was associated with a higher chance of discontinuation due to ineffectiveness.

This study was limited to the first treatment episode and the cumulative drug survival and its determinants would most likely be influenced when taking a second or third treatment episodes into account.

Our study consists of prospective real-world evidence data of 659 patients with up to 10 years of follow-up, allowing for the first time a drug survival analysis differentiated for different reasons of treatment discontinuation. The high effectiveness and safety of omalizumab has previously been demonstrated in clinical trials and small daily practice studies.[9, 58] Our study confirms the high effectiveness and safety of omalizumab by analysing drug survival per reason for discontinuation and demonstrating that the vast majority of patients discontinued treatment due to well-controlled disease and only a small proportion due to side-effects or ineffectiveness. The low drug survival rates due to ineffectiveness and side effects might be partially explained by the fact that no equivalent alternative treatment option is available, what might make patients and physicians restrained to discontinue omalizumab and accept some discomfort or subeffectiveness.

Only three previous studies, with small populations and short follow-up time, have evaluated the performance of omalizumab using drug survival analysis.[22, 53, 54]

Two earlier studies by our centre in an adult[53] and paediatric population[54], showed comparable drug survival rates related to well-controlled disease, side-effects and ineffectiveness. In line with our studies, Ke et al (n=298) found that around 72% of patients continued omalizumab treatment for at least 1 year and 60% after 18 months.[22] Since the data was obtained from medical and pharmacy claims, the authors could not differentiate between the reasons of discontinuation.

The large size and long follow-up period of our current multi-centre study, allowed analyses of determinants of omalizumab drug survival related to well-controlled disease, side-effects and ineffectiveness for the first time. Four determinants of omalizumab drug survival were found; fast response, the use of immunosuppressive drugs at start treatment, age, and a disease duration of two years or longer. Fast response resulted in a higher chance to discontinue treatment due to well-controlled disease and a lower chance to discontinue treatment due to side effects. In the last years, the available research data suggest that fast and/or good omalizumab response is pathomechanistically associated with the type I auto-allergy subtype and slow response with autoimmune subtype.[59, 60] Our data show that patients with fast response are more likely to enable to extend treatment intervals and discontinue treatment (thus having shorter drug survival due to well controlled disease), in contrast to patients with a delayed treatment response.[61] We further observed that patients with fast response reported significant less side effects compared to patients without fast response (n=62 vs n=84 respectively, p=0.038). Since patients with fast response had also significantly shorter omalizumab treatment duration (498 days 442 days, p=0.028), this could

partially explain the lower numbers of side-effects and subsequent discontinuation. Another reason for the lower number of reported side effects and lower chance to discontinue for that reason in patients with fast response might be that these patients are more likely to disregard side-effects due to earlier treatment satisfaction.

Another observation was that the use of immunosuppressive drugs at the start of omalizumab treatment determined a higher chance to discontinue treatment due to ineffectiveness. Similar to our study, three previous studies (n=48 to 154) report that previous immunosuppressive treatment was associated with poor treatment response.[21, 62, 63] In our population, immunosuppressive drugs (mainly corticosteroids) were prescribed before referral or used as short term course to bridge between the first visit at our hospital and start of omalizumab treatment in patients with severe disease. Usage of immunosuppressive drugs possibly reflects high disease severity in difficult to treat patients. These patients might have a lower chance of good response to omalizumab, leading to discontinuation due ineffectiveness. However, we did not observe a relation between baseline disease severity scores and discontinuation due to ineffectiveness, although baseline disease severity scores might have been biased for patients using immunosuppressive drugs. It remains unclear how immunosuppressive treatment before or at start omalizumab is related to (discontinuation due to) ineffectiveness and further research is needed to explain this finding.

A disease duration of two years or longer was identified to determine a lower chance to discontinue treatment due to well-controlled disease and a higher chance to discontinue treatment due to side effects and could be regarded as a unfavourable characteristic. A longer CU disease duration was previously associated with poor omalizumab treatment response in two studies although the effect on treatment duration for these patients remained unclear.[10, 63] In contrast, one study (n=85) found a significant shorter disease duration at baseline in non-responders (18 months) compared to partial (93 months) and complete responders (53 months).[64] However, our data show that a longer (>2 years) CU duration was associated with a lower chance of discontinuing treatment due to a well-controlled disease, but did not necessarily imply less effectiveness, since 68% showed partial or well-controlled disease under omalizumab treatment.

A disease duration of more than two years was also identified as determinant of a higher chance to discontinue due to side effects. Since only 22 patients discontinued treatment due to side effects, the reason for this remains unknown and warrants further investigation.

To date, this study offers the longest follow-up periods of omalizumab treatment in a patient population with CU. This allowed for detection of possible yet undiscovered, but relevant side-effects, since clinical trials often are limited to a follow-up period of several months. Side effects were reported relatively frequent (a total of 1235 by 71% of the patients (n=468)) within the population, however were generally mild with

fatigue (17%), headache (13%) and flu-like symptoms (11%). Hair loss appeared a notable side effect with 38 patients (6% of all patients with side effects) of which two patients discontinued omalizumab for this reason. Only four case reports previously described hair loss in six patients.[65-68] Only one case of anaphylaxis was reported as reason of treatment discontinuation in our study, resulting in a risk in our population of 0.15% in line with the previously, reported: 0.09%.[69]

In conclusion, well-controlled disease is the main reason of treatment discontinuation with fast response and short disease duration as favourable determinants. Immunosuppressive drug use at the start of treatment indicated a higher chance for treatment discontinuation due to ineffectiveness. These data provide important real-world practice information omalizumab performance and greatly supports managing expectations of patients and physicians when starting omalizumab as treatment for CU.

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SUPPLEMENTAL TABLES AND FIGURES

	Total n = 659	UMCU n = 357	EMC = 302
UCT (All)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
BL	4.0 (2.0-6.0) (n=479)	4.0 (2.0-7.0) (n=304)	3.0 (2.0-5.0) (n=175)
T1	10.0 (7.0-13.0) (n=432)	11.0 (7.0–14.0) (n=307)	8.0 (6.0-13.0) (n=125)
T3	12.0 (8.0-16.0) (n=348)	14.0 (9.0–16.0) (n=259)	10.0 (6.0-13.0) (n=89)
Tend	13.0 (8.0-16.0) (n=302)	14.0 (9.0-16.0) (n=244)	9.5 (5.8-14.0) (n=58)
UAS7 (Any wheals present group)	**Median (IQR). Patients with wheals: N=581**	**Median (IQR). Patients with wheals: N=330**	**Median (IQR). Patients with wheals: N=251**
BL	27.5 (20.3-34.0) (n=399)	27.0 (18.0–33) (n=231)	27.9 (21.1-34.9) (n=168)
T1	9.8 (2.0-20.3) (n=451)	8.0 (2.0-18.5) (n=287)	12.1 (2.1-22.4) (n=164)
T3	3.8 (0.0-12.7) (n=410)	3.0 (0.0-10.5) (n=255)	5.7 (0.0-14.0) (n=155)
Tend	0.0 (0.0-7.0) (n=388)	0.5 (0.0-7.0) (n=259)	0.0 (0.0-6.0) (n=129)
AAS (Any AO present group)	**Median (IQR). Patients with AO: N=343**	**Median (IQR). Patients with AO: N=204**	**Median (IQR). Patients with AO: N=139**
BL	33.8 (13.6-57.0) (n=98)	31.3 (13.8-51.0) (n=67)	48.0 (13.3-69.0) (n=31)
T1	2.5 (0.0-14.5) (n=136)	2.5 (0.0-13.6) (n=108)	2.5 (0.0-28.8) (n=28)
T3	0.0 (0.0-4.1) (n=125)	0.0 (0.0-3.6) (n=108)	0.0 (0.0-8.5) (n=17)
Tend	0.0 (0.0-0.0) (n=88)	0.0 (0.0-0.0) (n=84)	0.0 (0.0-11.3) (n=4)

Supplemental table S1. Disease activity scores per centre. BL: Baseline; T1: Second administration (four weeks after BL); T3: Fourth administration (three months after BL); Tend: Disease activity corresponding to the final dose or at data lock.

Type	Total	UMCU	EMC	Frequency of reason for discontinuation[a]	Reason for discontinuation UMCU[b]	Reason for discontinuation EMC[b]
All side-effects[c]	**1235 (100%)**	827 (100%)	408 (100%)	**22 (100%)**	13 (100%)	9 (100%)
Fatigue	**213 (17%)**	151 (18%)	62 (15%)	**5 (22%)**	4 (31%)	1 (11%)
Headache	**162 (13%)**	126 (15%)	36 (9%)	**2 (9%)**	1 (8%)	1 (11%)
Flu-like symptoms	**132 (11%)**	97 (12%)	35 (9%)	**1 (5%)**	1 (8%)	0 (0%)
Injection site reaction	**100 (8%)**	87 (11%)	13 (3%)	**0 (0%)**	0 (0%)	0 (0%)
Arthralgia	**92 (7%)**	66 (8%)	26 (6%)	**2 (9%)**	2 (15%)	0 (0%)
Hair loss	**38 (3%)**	30 (4%)	8 (2%)	**2 (9%)**	2 (15%)	0 (0%)
Unknown/not specified	**33 (3%)**	33 (4%)	0 (0%)	**0 (0%)**	0 (0%)	0 (0%)
Nausea	**53 (4%)**	31 (4%)	22 (5%)	**0 (0%)**	0 (0%)	0 (0%)
Dizziness	**53 (4%)**	30 (4%)	23 (6%)	**2 (9%)**	1 (8%)	1 (11%)
Myalgia	**41 (3%)**	37 (4%)	4 (1%)	**0 (0%)**	0 (0%)	0 (0%)
Gastrointestinal	**45 (4%)**	20 (2%)	25 (6%)	**1 (5%)**	0 (0%)	1 (11%)
Weight gain	**42 (3%)**	7 (1%)	35 (9%)	**0 (0%)**	0 (0%)	0 (0%)
Cardiovascular	**34 (3%)**	16 (2%)	18 (4%)	**0 (0%)**	0 (0%)	1 (11%)
Tingling sensation	**37 (3%)**	9 (1%)	28 (7%)	**0 (0%)**	0 (0%)	0 (0%)
Psychological	**25 (2%)**	22 (3%)	3 (1%)	**0 (0%)**	0 (0%)	0 (0%)
Dyspnoea	**15 (1%)**	15 (2%)	0 (0%)	**0 (0%)**	0 (0%)	0 (0%)
Skin symptoms	**15 (1%)**	7 (1%)	8 (2%)	**0 (0%)**	0 (0%)	0 (0%)
Temperature swings	**16 (1%)**	4 (<1%)	12 (3%)	**0 (0%)**	0 (0%)	0 (0%)
Urticaria flare-up	**18 (1%)**	3 (<1%)	15 (4%)	**2 (9%)**	0 (0%)	2 (22%)
Anaphylactic reaction	**1 (<1%)**	0 (0%)	1 (<1%)	**1 (5%)**	0 (0%)	1 (11%)
Other	**70 (6%)**	36 (4%)	34 (8%)	**5 (23%)**	2 (15%)[d]	1 (11%)[e]

Supplement table S2. Total reported side effects and side effects reported as reason for treatment discontinuation in the first treatment episode.
380 of the 529 patients (72%) reported at least one adverse event during treatment. [a] Percentage as proportion of all adverse events. [b]Percentage as proportion reported adverse events per centre. [c]One patient could report multiple adverse events. [d] Memory loss; Lymphedema [e]Neuralgia in leg.

	Total n=659			
Drug survival	**Overall**	**well controlled disease**	**Side effects**	**Ineffectiveness**
1 year	75%	85%	97%	94%
2 year	57%	69%	96%	92%
3 year	48%	61%	96%	92%
4 year	42%	56%	95%	91%
5 year	37%	50%	95%	90%
6 year	35%	49%	95%	90%

Supplemental table S3. Overall drug survival and drug survival differentiated for reason for discontinuation during the first treatment episode. Reasons for discontinuation: WCD (well-controlled disease), side effects and ineffectiveness

		Univariate		Multivariate	
Variabele[a]	**Number of patients (Total n=659)**	**Hazard ratio (CI interval)[b]**	**P-value**	**Hazard ratio (CI interval)**	**P-value**
Gender (Female)	Yes=474 / No=185	1.021 (0.735-1.420)	0.900		
Disease duration before start omalizumab ≥ 2 years	**Yes=338 / No=304**	**0.620 (0.457-0.843)**	**0.002**	**0.531 (0.381-0.738)**	**0.000**
Disease duration before start omalizumab (per year)	N=115	1.001 (0.999-1.003)	0.230		
Age at start treatment continue (per year)	**N=172**	**1.008 (0.998-1.018)**	**0.129**	**1.010 (0.999-1.020)**	**0.065**
Age at start treatment ≥ 40 years	Yes=352 / No=307	1.046 (0.774-1.414)	0.770		
Concomitant autoimmune disease	Yes=139 / No=520	1.131 (0.783-1.634)	0.513		
Immunosuppressive drug use at start of treatment	Yes=198 / No=461	0.898 (0.647-1.247)	0.522		
Fast response	**Yes=259 / No=271**	**1.311 (0.949-1.811)**	**0.101**	**1.462 (1.047-2.041)**	**0.026**
Wheals as main or sub diagnosis	Yes=581 / No=78	1.123 (0.704-1.792)	0.625		
AO as main or sub diagnosis	Yes=343 / No=316	1.081 (0.800-1.461)	0.613		
CindU as main or sub diagnosis	**Yes=324 / No=335**	**0.689 (0.510-0.933)**	**0.016**		
Wheals as main diagnosis	**Yes=563 / No=96**	**1.341 (0.871-2.065)**	**0.183**		
AO as main diagnosis	Yes=49 / No=610	0.781 (0.434-1.404)	0.408		
CindU as main diagnosis	Yes=47 / No=612	0.750 (0.417-1.348)	0.336		

Supplemental table S4. Univariate and multivariate analysis for determinants of drug survival related to well-controlled disease. [a]Factor concerning hazard ratio noted within brackets. [b]Variables with p<0.200 (in bold) are included in multivariate analysis. AO: Angioedema, CindU: Chronic inducible urticaria, Fast response: UAS7-score ≤ 6 or UCT-score ≥ 12 within 4 weeks after the first administration.

		Univariate		Multivariate	
Variabele[a]	**Number of patients (Total n=659)**	**Hazard ratio (CI interval)[b]**	**P-value**	**Hazard ratio (CI interval)[c]**	**P-value**
Gender (Female)	Yes=474 / No=185	1.336 (0.648-2.878)	0.412		
Disease duration before start omalizumab ≥ 2 years	Yes=338 / No=304	1.107 (0.587-2.088)	0.753		
Disease duration before start omalizumab (per year)	N=407	1.002 (0.997-1.006)	0.422		
Age at start treatment continue (per year)	N=657	0.998 (0.978-1.019)	0.883		
Age at start treatment ≥ 40 years	Yes=352 / No=307	0.715 (0.381-1.341)	0.296		
Concomitant autoimmune disease	Yes=139 / No=520	1.370 (0.667-2.814)	0.392		
Immunosuppressive drug use at start of treatment	**Yes=198 / No=461**	**1.885 (1.003-3.541)**	**0.049**	**1.885 (1.003-3.541)**	**0.049**
Wheals as main or sub diagnosis	Yes=581 / No=78	0.798 (0.334-1.905)	0.611		
AO as main or sub diagnosis	Yes=343 / No=316	1.232 (0.651-2.332)	0.522		
CindU as main or sub diagnosis	**Yes=324 / No=335**	**0.595 (0.312-1.137)**	**0.116**		
Wheals as main diagnosis	Yes=562 / No=96	0.915 (0.404-2.075)	0.832		
AO as main diagnosis	Yes=49 / No=610	1.234 (0.439-3.474)	0.690		
CindU as main diagnosis	Yes=47 / No=612	0.287-3.028 (0.932)	0.906		

Supplemental table S5. Univariate analysis for determinants of drug survival related to ineffectiveness. [a]Factor concerning hazard ratio noted within brackets. [b]Variables with p<0.200 (in bold) are included in multivariate analysis. AO: Angioedema, CindU: Chronic inducible urticaria, Fast response: UAS7-score ≤ 6 or UCT-score ≥ 12 within 4 weeks after the first administration.

		Univariate		Multivariate	
Variabele[a]	**Number of patients (Total n=659)**	**Hazard ratio (CI interval)[b]**	**P-value**	**Hazard ratio (CI interval)[c]**	**P-value**
Gender (Female)	Yes=474 / No=185	1.785 (0.604-5.274)	0.295		
Disease duration before start omalizumab ≥ 2 years	**Yes=338 / No=304**	**2.804 (1.026-7.661)**	**0.044**	**3.443 (1.131-10.480)**	**0.030**
Disease duration before start omalizumab (per year)	N=391	1.002 (0.997-1.006)	0.422		
Age at start treatment continue (per year)	N=635	0.993 (0.966-1.021)	0.618		
Age at start treatment ≥ 40 years	Yes=352 / No=307	0.483 (0.202-1.151)	0.100		
Concomitant autoimmune disease	Yes=139 / No=520	1.440 (0.563-3.681)	0.447		
Immunosuppressive drug use at start of treatment	Yes=198 / No=461	1.284 (0.539-3.063)	0.572		
Fast response	**Yes=259 / No=271**	**0.173 (0.050-0.593)**	**0.005**	**0.165 (0.48-0.570)**	**0.004**
Wheals as main or sub diagnosis	Yes=581 / No=78	2.937 (0.395-21.842)	0.293		
AO as main or sub diagnosis	Yes=343 / No=316	1.517 (0.636-3.616)	0.348		
CindU as main or sub diagnosis	Yes=324 / No=335	0.560 (0.234-1.337)	0.192		
Wheals as main diagnosis	Yes=563 / No=96	1.888 (0.441-8.080)	0.392		
AO as main diagnosis	Yes=49 / No=610	1.120 (0.262-4.794)	0.879		
CindU as main diagnosis	Yes=47 / No=612	0.044 (0.000-45.068)	0.377		

Supplemental table S6. Univariate analysis for determinants of drug survival related to side-effects. [a]Factor concerning hazard ratio noted within brackets. [b]Variables with p<0.200 (in bold) are included in multivariate analysis. AO: Angioedema, CindU: Chronic inducible urticaria, Fast response: UAS7-score ≤ 6 or UCT-score ≥ 12 within 4 weeks after the first administration.

CHAPTER 3

Safety and effectiveness of omalizumab for the treatment of chronic urticaria in pediatric patients

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ABSTRACT

Background: Evidence on safety and effectiveness of omalizumab for treatment of chronic urticaria in pediatric patients is scare and limited to case reports. In particular, drug survival of omalizumab has not yet been investigated, which is a key element in the evaluation of its clinical performance. The aim of this study is to investigate safety, effectiveness and drug survival rates of omalizumab in a daily practice cohort of pediatric patients with chronic urticaria.

Methods: This is a multicenter study including all pediatric patients from an academic center (Wilhelmina Children's Hospital) and a general center (Diakonessenhuis Hospital) in the Netherlands, who started omalizumab treatment before the age of 18 years. Data on safety, effectiveness, time to discontinuation and reasons for discontinuation of treatment were assessed. Drug survival of omalizumab was estimated using the Kaplan-Meier survival analysis.

Results: A total of 38 patients, who started treatment between January 2014 and January 2020, were included. Most patients (68.4%) used omalizumab without reporting any side-effects and a complete or good response to treatment was achieved in 76.3% of patients. The 1- and 2-year drug survival rates were 62% and 50% respectively, with well-controlled disease activity as the most frequent reason for discontinuation in 69.2% of patients, followed by ineffectiveness in 23.1% and side-effects in 7.7% of patients.

Conclusions: This study demonstrates high safety and effectiveness of omalizumab treatment in pediatric patients with chronic urticaria, which will aid clinical decision making and management of expectations when choosing omalizumab treatment for pediatric patients with CU.

INTRODUCTION

Chronic urticaria (CU) has a high prevalence in the pediatric population, estimated around 0.5%[1,2], and affects the psychological state and quality of life considerably.[2] Current treatment guidelines recommend a stepwise approach that aims at total symptom control. In CU patients with insufficient response to four-times the standard dose of H1 anti-histamines, add-on therapy should be considered.[2]

Omalizumab, a monoclonal antibody against immunoglobulin E[3], is recommended as add-on treatment in adults and adolescents (≥12 years) with CU. Studies on the safety and efficacy of omalizumab for the treatment of CSU, however, were mainly performed in adult patient populations in three randomized controlled trials (RCTs) that included 5, 18 and 10 adolescents only.[4–6] Although none of the studies reported specific outcomes of the use of omalizumab in adolescents, overall, omalizumab achieved a statistically significant reduction in disease-severity scores and was well tolerated.[4–6] In addition, several case reports including a total of fifteen children aged 4 to 16 years describe safe and successful use of omalizumab.[7–12] Although studies on safety and efficacy of omalizumab in children with CU are scarce, the clinical safety and efficacy of omalizumab in the pediatric population has been demonstrated in large, RCTs in patients with asthma aged 6 to 12 [13–16] and 6 to 20 years (n=246-627).[17]

Drug survival of drug therapy is a key element in the evaluation of its clinical performance.[18] In short, drug survival is the length of time patients remain on a specific drug, investigated using the technique of survival analysis.[19] Survival analysis involves a series of statistical techniques to study time until the occurrence of an event of interest, in this case the discontinuation of omalizumab. By analyzing the treatment duration and reasons for discontinuation of treatment, drug survival analysis reveals the drug's safety, tolerability and effectiveness over time, as well as patients' and doctors' preferences. Therefore, drug survival can be used as an indicator of therapeutic success in daily practice.[19]

Recently, the drug survival of omalizumab has been studied in an adult Dutch CU population.[20] This study showed a 1-, 2- and 3-year overall drug survival of omalizumab of 77%, 61% and 55%, respectively, mostly determined by well-controlled disease activity, demonstrating high safety and effectiveness in daily practice.[20] In pediatric patients with CU, however, daily practice data of treatment with omalizumab is lacking. Furthermore, the drug survival of omalizumab in pediatric patients with CU has not yet been investigated. The primary aim of this study is to investigate safety and effectiveness of omalizumab in a daily practice cohort of pediatric patients with CU.

METHODS

Patient and data collection

All patients in the Wilhelmina Children's Hospital (WKZ) - part of the University Medical Center Utrecht and the Diakonessenhuis Hospital - a general hospital in Utrecht (Netherlands), who were diagnosed with CU and were prescribed omalizumab before the age of 18, were screened for inclusion.

All variables used in the study, regarding information on patient characteristics, treatment characteristics and disease activity, were extracted from the electronic patient record. CU disease activity was assessed using the Urticaria Activity Score 7 (UAS7)[2] The smallest difference between UAS7-scores with clinical relevance, the minimal important difference (MID), was defined as a decrease in the UAS7-score of ten points.[21]

Start- and stop data of omalizumab, response to treatment, side-effects and reasons for treatment discontinuation were captured. Only the first treatment episode with omalizumab was used for analyses. If omalizumab treatment was discontinued and restarted within a 90 day timeframe, the subsequent dosage was considered part of the initial treatment episode; otherwise it was considered as new treatment episode .

All patients were treated according to the local treatment protocol based on the international guidelines.[22] Briefly, treatment with omalizumab was initiated in children with high disease activity scores (UAS7 ≥ 16) despite the use of antihistamines in fourfold the licensed dose for children. Initial treatment consisted of 300 mg omalizumab every four weeks, with an increase to 450 mg or 600 mg in case of insufficient treatment response (UAS7>16) after the 6th administration. If treatment was effective, a down-dosing schedule was initiated that entailed progressively increasing the dosing interval by 1 week every visit up to eight weeks.[23] Omalizumab was discontinued if good disease control was maintained after an interval of 8 weeks.

Treatment response was classified as "complete" - (UAS7-score = 0), "good" - (0<UAS7-score<7), "partial" - (UAS7-score >6; MID ≥10) or "poor" - (UAS7-score >16; MID <10) and was evaluated at second administration (T2) and at end of treatment or datalock. In case of missing activity scores, treatment response was determined by four different authors independently, based on the physicians' description in the patients' charts.

An assessment outcome of being symptom-free or being satisfied, with minor symptoms only, equals a complete and good response respectively, whereas the description of an insufficient or lacking response equals a partial or poor response respectively. Any discrepancies were resolved by consensus.

Statistical analyses

Statistical analyses were performed by using IBM SPSS Statistics (25.0.0.2). Comparisons of measures at different time points were made using a paired t-test or Wilcoxon-Signed rank test for continuous variables. Statistical significance was assumed for P-values ≤0.05.

Drug survival rates of omalizumab were calculated by the Kaplan-Meier method and displayed in survival curves for overall drug survival (event: discontinuation due to any reason) and survival curves for different reasons of discontinuation (events: well-controlled disease activity, ineffectiveness, side-effects or combination of side-effects and ineffectiveness). Patients who did not discontinue treatment at the time of datalock or were lost to follow-up, were censored. Data were compared with previously published data of drug survival in adult CU patients.[20] The Log rank (Mantel-Cox) test was used to compare the survival curves of adult and pediatric patients. To identify if determinants were associated with discontinuation of treatment, a univariate Cox regression model was performed. Those determinants with a P-value ≤0.2 were entered into a multivariate Cox regression model with backward selection. In the multivariate Cox regression model, determinants with a P-value ≤0.05 were considered statistically significant.

This study was approved by the Medical Ethic Review committee in both participating centers [protocol number 19-808/C]. Exemption regarding obtaining informed consent was granted according to the GDPR. Analyses were performed with completely pseudonymized data.

RESULTS

Patient and treatment characteristics

Of 56 screened patients, eighteen were excluded because: (i) they received omalizumab for indications other than CU (N=14); (ii) treatment was never initiated (N=2); or (iii) the initial diagnosis of CU was corrected after start of treatment (N=2). In total, 38 patients (57.9% female), who started treatment between January 2014 and January 2020, were included from the two centers - Wilhelmina Children's Hospital (n=28) and Diakonessenhuis Hospital (n=10).

Patient characteristics are shown in Table 1. The median age of all patients at start of omalizumab was 14.9 years with the youngest patient being 3.6 years and the oldest patient being 17.7 years. Six patients (15.8%) were younger than 12 years at start of treatment. The majority of patients (76.3%) presented mainly with spontaneous wheals, whereas 17.5% and 5.0% presented with chronic inducible urticaria (CIndU) and angioedema (AE) respectively. Based on self-reported triggers, the CIndU patients had cold urticaria (n=3), cholinergic urticaria (n=2) and symptomatic dermographism (n=2). In total, 32 patients experienced multiple components of CU. Omalizumab treatment was

initiated a median of 20.5 months after the first onset of CU. There were no differences between the patients from the two participating centres.

The majority of patients (92.1%) were treated with antihistamines throughout omalizumab treatment; the majority being treated with antihistamines four times a day. Besides antihistamines, montelukast and corticosteroids were used simultaneously during omalizumab treatment in 7.9% and 2.6% of patients respectively. Detailed treatment characteristics are shown in supplemental Table 1.

	Total
Gender (female)	22 (57.9%)
Age at start Omalizumab (years), median (IQR)	14.9 (12.9 – 16.0)
Age at start Omalizumab (categorized)	
<6 years	1 (2.6%)
6 to 12 years	5 (13.2%)
12 to 16 years	20 (52.6%)
16 to 18 years	12 (31.6%)
Main diagnosis	
CSU	**29 (76.3%)**
CSU with AE	17
CSU with CIndU	5
CSU with AE and CIndU	3
AE	**2 (5.3%)**
AE with CIndU	1
CIndU	**7 (18.4%)**
CIndU with CSU	3
CIndU with AE	3
Auto-immune disease †	2 (5.3%)
Duration of CU at start of Omalizumab (months), median (IQR) ‡	20.5 (12.5 – 48.2)
Duration of CU at start of Omalizumab (categorized)	
< 1 year	9 (23.7%)
1 to 2 years	12 (31.6%)
2 to 5 years	9 (23.7%)
5 to 10 years	8 (21.1%)

Table 1: Patient characteristics
†One patient (male, 16.8 years old) suffered from diabetes mellitus type 1 and one patient (female, 14.9 years old) suffered from diabetes mellitus type 1 and hypothyroidism
‡Duration of CU estimated in 18 patients (47.4%)

Half of the patients (50.0%), of whom two patients restarted treatment within 90 days after discontinuation, were continuously treated with omalizumab. In total, 13 patients

(34.2%) discontinued omalizumab treatment, of whom two patients restarted treatment after 212 and 226 days. Six patients (15.8%) were lost to follow-up. The median post-treatment follow-up was 18.5 months (min. 1.2 months; max. 72.5 months).

The UAS7-scores at baseline and end of treatment were available for 20 children and the median score decreased significantly from 25.5 at baseline to 0.60 at end of treatment (p=0.0001). At end of treatment or at datalock, treatment response was based on UAS7-scores in 26 patients (68.4%) and on the physicians' documentation in 12 patients (31.6%). Overall, most patients were complete responders (39.5%) or good responders (36.8%), of which 36.8% of patients had achieved this response at the second administration. Ultimately, six patients (15.8%) were partial responders and three patients (7.9%) were poor responders. Response to omalizumab in CSU and/or AE patients and CindU patients was comparable with 77.4% respectively 71.4% of patients achieving a complete or good response (p=0.850). Among the patients who presented with CIndU, response was partial or lacking in only two patients in whom their CIndU was triggered by cold.

Almost all patients (97.4%) started omalizumab treatment with a dose of 300 mg. Throughout omalizumab treatment, 300 mg was the maximum dose in 71.1% of patients. Ten patients, aged between 12 and 16.8 years, received a higher dose: six patients (15.8%) and four patients (10.5%) were treated with a dose of respectively 450 mg and 600 mg at least once. There were no differences in the specific patient characteristics gender, age, BMI, disease duration and disease activity at start of treatment between patients treated with standard and high dose of omalizumab.

Side-effects

In total, twelve children reported one or more side-effects. The most frequent reported side-effect was headache (n=5, 41.7%). Other reported side-effects were fatigue (n=2, 16.8%), flu like symptoms (n=1, 8.3%), injection site reaction (n=1, 8.3%), arthralgia/ joint pain (n=1, 8.3%) and hair loss (n=1, 8.3%). None of these side-effects led to discontinuation of omalizumab. The parents of one patient (8.3%) attributed loss of concentration due to omalizumab treatment. This was the reason for discontinuing omalizumab after six administrations in this patient. These observations are presented in Table 2. The use of higher doses of omalizumab (450 and 600mg) was not associated with an increase of side-effects.

Patient	Sex	Age†	No. of administrations	Maximum dose during treatment (no.)	Most important reported side-effect	Additional reported side-effects	Reason for discontinuation?
1	F	10.6	11	300 mg	Headache	Fatigue, dizziness, injection site reaction	No
2	M	8.5	12	300 mg	Injection site reaction		No
3	F	17.5	8	300 mg	Headache	Fatigue, nausea, dizziness	No
4	F	16.8	11	300 mg	Arthralgia/ joint pain	Headache, flu like symptoms	No
5	F	15.4	12	450 mg (1)	Hair loss		No
6	F	11.1	19	300 mg	Fatigue	Arthralgia/ joint pain	No
7	F	15.9	30	300 mg	Fatigue	Headache, itchy arms	No
8	F	14.9	8	300 mg	Headache	Stomach ache, nausea	No
9	F	14.7	4	300 mg	Flu like symptoms		No
10	M	13.4	11	450 mg (3)	Loss of concentration		Yes
11	F	16.3	25	300 mg	Headache		No
12	M	12.9	3	300 mg	Headache		No

Table 2: Side-effects during omalizumab treatment
†Age (years) at start of omalizumab treatment

Drug survival

Overall, 13 (34.2%) patients discontinued treatment. The most frequent reason for discontinuation of omalizumab was well-controlled disease activity in nine (69.2%) patients. Other reasons for treatment discontinuation were ineffectiveness in three patients (23.1%) and side-effects in one patient (7.7%).

The 1- and 2-year overall drug survival was 62% and 50% respectively, mostly determined by well-controlled disease activity (Figure 1). The overall drug survival rates of pediatric patients and adult patients[20] were not significantly different (p=0.227). However, the drug survival curves related to discontinuation due to well-controlled disease activity – which were compared since this was the most frequent reason of

treatment discontinuation in both adult- and pediatric patients – differed significantly (p=0.032, supplemental Figure 1).

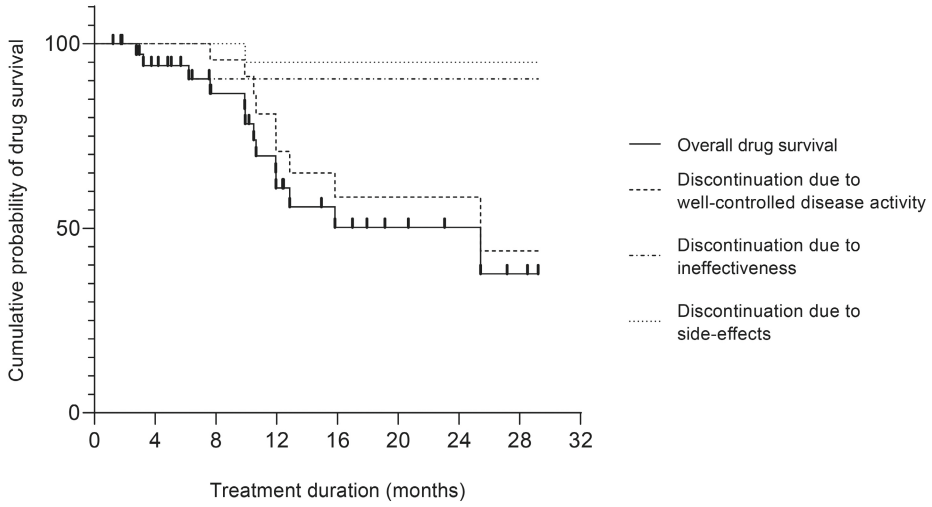

Figure 1: Drug survival of omalizumab in pediatric patients

Determinants of drug survival were analyzed by Cox regression analysis (Table 3). None of the factors, including age at start of omalizumab treatment, gender, disease duration, disease activity at baseline or the main diagnosis, appeared to affect discontinuation of treatment. No factors were entered into a multivariate Cox regression.

	Hazard ratio [95% CI]	P-value
Age at start of omalizumab treatment	1.06 [0.88-1.26]	0.55
Gender	2.03 [0.63-6.55]	0.24
Disease duration	0.99 [0.97-1.01]	0.30
Disease activity at baseline	1.04 [0.97-1.11]	0.26
CIndU†	0.58 [0.12-2.67]	0.48
Angioedema†	1.05 [0.13-8.33]	0.96

Table 3: Univariate Cox-regression: determinants of drug survival in pediatric patients with CU. Data are presented as hazard ratio with 95% Confidence Interval.
†Patients with CIndU respectively Angioedema compared to patients with CSU

DISCUSSION

This study represents the largest daily practice cohort study of pediatric patients with CU being treated with omalizumab to date. We provide long-term safety, effectiveness and drug survival data on omalizumab for treatment of chronic urticaria in pediatric, spanning a timeframe of six years. The safety profile of omalizumab was reassuring since most patients (68.4%) used omalizumab without reporting any side-effects and

only mild side-effects. The side-effects, mainly headache and fatigue, were reported by twelve patients (31.6%). The majority of patients (76.3%) achieved a good or complete response at end of treatment or datalock, whereas 15.8% and 7.9% of patients were partial and poor responders, respectively. Our results showed a 1- and 2-year drug survival of 62% and 50% respectively, mainly determined by well-controlled disease activity.

Previously published work on safety and effectiveness of omalizumab treatment in patients with CU is limited to a small number of adolescents (n=33) included in three RCTs[4–6] and fifteen pediatric patients aged 4 to 16 years[7–12] described in case reports. None of the RCTs presented specific outcomes from use of omalizumab in adolescents. All case reports describe a complete or good (UAS7≤6) response to omalizumab in 86.7% respectively 13.3% of patients, with a treatment duration varying from 3 to 20 months.[7–12] None of these patients reported any side-effects. However, the potential outcome reporting bias inherent to case reports and the lack of specific outcomes from use of omalizumab in adolescents in the RCTs, complicates the comparison of our data with published work.

Our results concur with the results derived from other studies in adult patients with CU. The effectiveness of omalizumab found in our study is comparable to daily practice studies in adult patients, with complete and good response rates varying between 64% and 83%.[20,24–26] In addition, the overall 1- and 2-year drug survival rates found in this pediatric population are not statistically different from previously published drug survival rates in adult patients with CU.[20] However, the drug survival curves related to discontinuation due to well-controlled disease activity in pediatric and adult patients were statistically different (p=0.032), suggesting that pediatric patients are more likely to discontinue treatment due to well-controlled disease activity compared to adult patients. This, however, needs to be investigated in larger studies.

Safety outcomes of our study are comparable to outcomes of both pediatric patients with asthma and adult patients with CU, who are treated with omalizumab. Three RCTs in pediatric patients with asthma demonstrated well-tolerated and safe use of omalizumab, with discontinuation due to side-effects in 0.4% to 1.2% of patients whereas side-effects were reported in up to 93.4%.[14–16] In these RCT's, pediatric patients with asthma reported (naso)pharyngitis, sinusitis and upper respiratory tract infection as most-common side-effects.[14–16] Headache, which was the most frequently reported side-effect in our study, was mentioned in 13.8% to 36.0% of patients.[14–16] Real-world data in pediatric patients with asthma suggest higher rates of discontinuation due to side-effects, with fatigue as the most frequently reported side-effect.[27,28] This is comparable to earlier results, where headache and fatigue were the most frequently reported side-effects in adult patients with CU.[29] The percentage of patients using a dose of omalizumab higher than 300 mg in our study (26.3%) was very similar to the

percentage recently published in adult patients (27.0%).[30] In addition, similar to our results, patient characteristics did not differ significantly between patients treated with standard or higher dose.[31] Interestingly, in both pediatric and adult CU patients, the incidence of side-effects during high dose treatment was not elevated compared to standard treatment. Loss of concentration, which was the only side-effect that led to discontinuation in our study (in one patient), was mentioned in neither RCTs nor real-world data of pediatric patients with asthma or adult patients with CU.

Although being the largest daily practice cohort of pediatric patients treated with omalizumab published to date, the small number of patients remains a limitation of this study. Due to this limitation, no determinants for drug survival could be analyzed in a multivariate Cox-regression model. However, this cohort was recruited from one academic and one general hospital in the Netherlands, where the largest number of pediatric patients are treated. Hence, this cohort is likely to have high external validity. In addition, we believe an accurate representation of the real-world use of omalizumab could be rendered through detailed and complete collection of data. Nevertheless, our study accentuates the need for additional, larger-scale studies to investigate omalizumab treatment in this population.

In conclusion, our study demonstrates high safety and effectiveness of omalizumab in pediatric patients with CU. We have shown that omalizumab has a good overall survival rate, with well-controlled disease activity being the main reason for discontinuation, confirming the safe and effective use of omalizumab in this population. These findings will aid clinical decision making and management of expectations when choosing omalizumab treatment for pediatric patients with CU.

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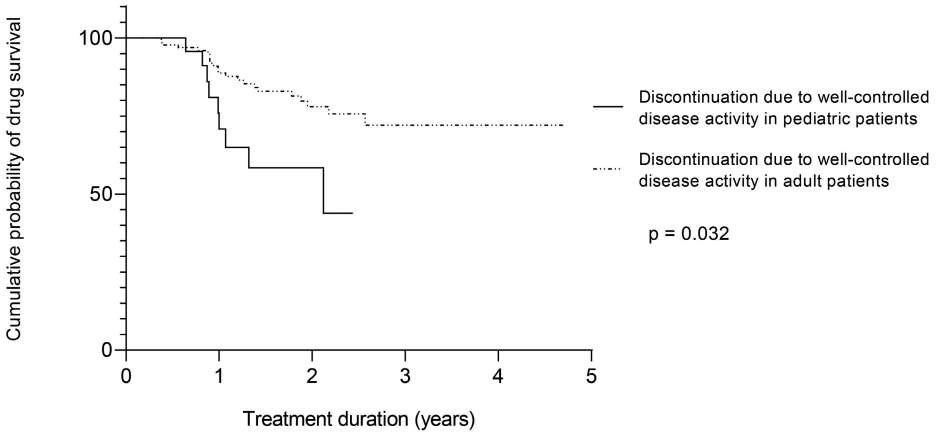
SUPPLEMENTAL TABLES AND FIGURES

	Total
Maximum dose of omalizumab during treatment	
150 mg	1 (2.6%)
300 mg	27 (71.1%)
450 mg	6 (15.8%)
600 mg	4 (10.5%)
Discontinuation of omalizumab, no (%)	13 (34.2%)
Use of antihistamines during Omalizumab	
Yes, 4dd	18 (47.4%)
Yes, but lower than 4dd	14 (36.8%)
Yes, but higher than 4dd	3 (7.9%)
No antihistamine	3 (7.9%)
Other comedication during omalizumab	
Montelukast	3 (7.9%)
Corticosteroids	1 (2.6%)
Other*	6 (15.8%)
Post-treatment follow-up,** median (IQR)	18.5 (7.6 – 33.45)

Supplemental Table 1: Characteristics of treatment with omalizumab and comedication during omalizumab treatment
*Tranexamic acid (N=1), the combination of levetiracetam, clobazam and cannabis oil (N=1) and methylphenidate (N=3)
** Post-treatment follow-up is calculated by the date of datalock minus date of start of omalizumab



Supplemental Figure 1: Comparison of drug survival curves in pediatric and adult patients related to discontinuation due to well-controlled disease activity
Data of drug survival in adult patients originate from the study of Spekhorst et al.[21]

CHAPTER 4

High dose omalizumab use in patients with chronic spontaneous urticaria.

J Allergy Clin Immunol Pract 2020 Apr;8(4):1426-1427.e1.

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TO THE EDITOR:

Omalizumab, a monoclonal anti-IgE antibody, is an effective and safe treatment option in patients with Chronic Spontaneous Urticaria (CSU) who respond poorly to fourfold standard dose antihistamines.[70] The recommended dose of omalizumab is 300mg every four weeks.[70] In our centre, patients with insufficient response to 300mg omalizumab are administered with an increased dose. However, the effectiveness thereof is insufficiently investigated. The objective of this retrospective daily practice cohort study was to assess the effectiveness and safety of the referred-to treatment strategy.

Data of all patients diagnosed with CU (Chronic urticaria) and treated with omalizumab in our department (February 2012–October 2018) were collected. According to the drug label and International and Dutch guidelines[70, 71], all patients were initially treated with omalizumab 300mg every four weeks. Based on our local protocol, to patients with insufficient response (UAS7>16; UCT< 11 and/or moderate to severe angioedema) after 5 injections, updosing is offered. Subsequently, the omalizumab dose was increased to 450mg or 600mg every four weeks. If treatment response was still insufficient after three doses of 600mg omalizumab, treatment interval could be shortened to two weeks. Omalizumab was discontinued in cases where two consecutive doses of 600mg at two weeks interval yielded insufficient response.

Treatment response was assessed using urticaria disease activity scores, UAS7 and UCT, [72] before the first administration (baseline-T0), before updosing omalizumab (baseline-HD) and at the end of high dose (HD) treatment (T-end-HD). Response was defined as improvement of disease activity by a minimal important difference (MID) of 10 UAS7 points or, if UAS7 was not available, 3 UCT points.[56, 57] Complete response was defined as UAS7 = 0 or UCT = 16. Treatment response and side effects were compared before and after updosing omalizumab.

This study was approved by the Medical Ethic Review committee (METC-Utrecht-number 18-557).

A total of 166 patients (mean age 42 years; 73% female) were treated with omalizumab. 122 patients (73%) received a standard dose (300mg) and 44 patients (27%) a higher dose of 450 mg (n=11) or 600mg (n=33). In nine patients treatment interval was additionally shortened to 3 or 2 weeks during high dose treatment, resulting in an effective dose higher than 600mg every 4 weeks. Patient and treatment characteristics did not differ significantly between patients treated with standard and high dose omalizumab (Table E1). All patients confirmed compliance to antihistamines 4 times a day at start and during high dose treatment.

Higher doses of omalizumab were introduced after a mean of 8.3 administrations of standard dose (IQR;5-9). UAS7 scores showed a significant improvement on comparing UAS7 before dose increase (median 20.0; IQR 10.5-26.0) to end of high dose treatment (median 4.3; IQR 0-14.0) (p<0.0001) (Figure 1).

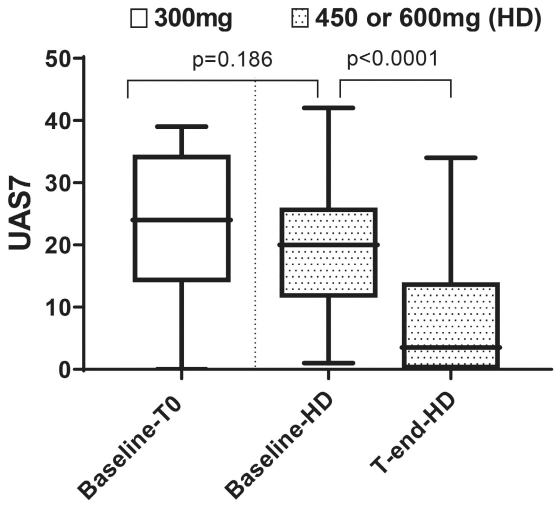

Figure 1 Urticaria activity in patients with high dose treatment
Legend: Urticaria activity based on USA7 scores of patients receiving high dose treatment (n=44) is presented.
white box-plot:
- Baseline-T0: before start omalizumab 300mg; median UAS7= 24 (IQR: 14.0-34.50)
dotted box-plot:
- Baseline-HD: before start high dose (450 or 600mg); median UAS7 = 20 (IQR: 11.25-26.0)
- T-end-HD: at the ends of high dose treatment; median UAS7 = 3.3 (IQR: 0.0-14.0)
If symptom scores were missing, data of a maximum of four weeks away were used. Missing UAS7 scores at Baseline, Baseline-HD and T-end-HD respectively: n=15, n=7, n=6.

Treatment response after updosing compared to standard dose based on UAS7 scores could be calculated for 41 of the 44 patients (table 1). Three patients with missing UAS7 and UCT score were classified as non-responders, based on information from their individual records. 61% (n=27) of the patients showed an improved treatment response after updosing, of which 32% (n=14) showed a complete and 30% (n=13) a partial response, while 17 patients did not improve upon updosing. Seven patients became complete responders on high dose were non responders on standard dose, five complete responders were partial responders on standard dose and in two cases of complete response, exact data regarding treatment response on standard dose was missing.

treatment response based on UAS7 and UCT scores[#]	Standard dose	High dose
Complete responders	0(0%)	14(32%)
Partial responder	13(30%)	13(30%)
Non responder	23(52%)	14(32%)
Missing	8[ǯ](18%)	3*(7%)

Table 1 Treatment response to standard dose and high dose treatment
Legend: Clinical response in patients treated with high dose omalizumab (n=44) is presented.
Response to standard dose (300 mg) is determined by comparing UAS7 score between baseline-T0 and baseline-HD (before up dosing).
Response to high dose is determined by comparing UAS7 score between baseline-HD and T-end-HD. complete response: UAS7 = 0; partial response: minimum reduction of 10 UAS7 points; non response: no UAS7 reduction or less than 10 UAS7 points.
If UAS7 score was missing, response was calculated using UCT;
complete response: UCT = 16; partial response: minimum raise of 3 UCT points; non response: no UCT raise or less than 3 UCT points.
[#]The UCT score was used to determine treatment response for 10 patients for standard dose treatment and for 4 patients for high dose treatment.
[ǯ] Patient records of eight patients with missings were studied, no valid estimation of response could be made.
* 3 patients without clinical activity scores showed no treatment response according to their patient record and were defined as non-responders.

Analysis, including multiple testing correction, of all available baseline variables showed no significant predictors of response to high dose omalizumab. In nine patients with interval frequency shortening during high dose (600mg) omalizumab treatment, no additional clinical improvement was observed. The high dose non-responder group (n=17) received a median of 3 doses (IQR: 1-12.5) high dose omalizumab before stopping treatment.

While high dose omalizumab treatment up to 600mg / 2 weeks, based on body weight and total IgE, is a regularly applied in asthma patients,[73] this therapeutic approach is rarely applied in CU.

A small number of recent studies with lower patient numbers, describing the strategy of omalizumab updosing in CSU patients, showed a comparable percentage (23%) of patients using higher doses[12, 13] as observed in our study and also described the effect of updosing omalizumab with complete or partial responses ranging from 45%-83%.[12-14] These were, however, analysed by comparing disease activity before starting omalizumab treatment and at the end of high dose treatment, which was comparable to 72% in our population. Importantly, we analysed the additional effect of updosing by comparing effect of standard dose to high and observed relevant improvement in 61% of patients which had not been depicted before. Additional updosing of omalizumab by interval shortening, did not show clinically relevant improvement. However higher patient numbers are needed to validate this initial finding.

The most frequently reported side effects during high dose treatment were headache, fever like sensations and tiredness, which were not increased compared to standard treatment. Likewise, higher doses of omalizumab are well tolerated in asthma patients.[73]

In conclusion, updosing omalizumab led to an additional clinical improvement in 61% of the patients without reporting additional side effects. Further studies are needed to determine the best strategy for updosing omalizumab in patients who do not respond to the standard treatment of CSU.

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CHAPTER 5

Effective omalizumab interval prolongation in the treatment of chronic urticaria.

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TO THE EDITOR:

Omalizumab, a monoclonal anti-IgE antibody, is an effective and safe add-on treatment option in patients with Chronic Spontaneous Urticaria who respond poorly to fourfold standard dose antihistamines.[70] The recommended treatment dose of omalizumab is 300mg every four weeks. In daily practice, interval shortening is used to intensify treatment in less responsive cases, while interval prolongation is aimed at discontinuing treatment.[14, 74]

A few studies with small patient numbers (varying between 7 and 20) investigated the possibility of increasing omalizumab treatment intervals in patients with active Chronic Urticaria (CU).[16-18, 75] However, no data are available from a large CU population. The objective of our study was to investigate the maximum treatment interval while maintaining well-controlled disease status in patients with active CU.

Data (February 2012 – October 2019) concerning patient and treatment characteristics of all patients with chronic urticaria on omalizumab treatment in our department were collected. According to the SMPC, International, and Dutch guidelines,[70, 71] all patients were initially treated with omalizumab 300mg every four weeks. After six administrations, treatment intervals in patients with well-controlled disease (UAS7≤6 or UCT≥12), were gradually increased by one week. Treatment was discontinued in patients with well-controlled disease at an 8-week treatment interval. Based on a shared decision between patient and physician, continuous treatment with intervals longer than 8 weeks was possible.

When symptoms reoccurred during an extended treatment interval, the interval was shortened to the last symptom-free interval.

To identify the individual maximum omalizumab interval, we determined each patient's steady state interval. This is defined as the longest well-controlled (UCT ≥12 or UAS7 ≤6) treatment interval which a patient achieved on at least two consecutive administrations. This interval was allowed to be interrupted once by a longer unsuccessful interval (i.e. recurring symptoms) if the patient subsequently returned to a symptom-free steady state interval. To eliminate possible bias due to a short treatment period, patients with a follow-up period (including treatment period) of sixteen months or shorter were excluded from analyses. This timeframe was chosen to allow patients to finish the minimal treatment period of 12.5 months and a follow-up period of two times 8 weeks (steady state).

238 patients (mean age 41 years; 71% female) were screened. 106 patients were under treatment for less than sixteen months and were thus excluded. Of the remaining 132 patients: a) 38 patients (29%) discontinued omalizumab due to well-controlled disease

without the need to restart treatment (WCD-stop group); b) 26 patients (20%) initially discontinued omalizumab due to well-controlled disease, but later restarted treatment (RS group); c) 58 patients (44%) had well-controlled disease under continuous treatment (CT group) ; d) 10 patients (8%) discontinued omalizumab due to poor response to treatment (PR group). For the RS group, treatment episodes were differentiated into first treatment episode, before discontinuing omalizumab (RS1), and second treatment episode, after restarting omalizumab (RS2).

Patient characteristics are presented in table E1 and were comparable to other daily practice populations. [9] Percentages of patients reaching a specific steady state treatment interval are presented in table 1. Of the total population analysed, 73% of patients were able to extend the treatment interval to a steady state interval of six weeks or longer, while 57% of patients were able to extend the interval to a steady state interval of eight weeks or longer. Only 18% of the patients with response to treatment were unable to extend the interval beyond four weeks.

	Total study population	Active disease	RS2	CT
	n=132	n=84	n=26	n=58
3 weeks or more	121(92%)	76(91%)	19(73%)	57(98%)
4 weeks or more	119(90%)	74(88%)	19(73%)	55(95%)
5 weeks or more	108(82%)	63(75%)	19(73%)	44(76%)
6 weeks or more	96(73%)	50(60%)	18(69%)	32(55%)
7 weeks or more	87(66%)	38(45%)	15(58%)	23(40%)
8 weeks or more	75(57%)	21(25%)	10(39%)	11(19%)
9 weeks or more	-*	10(12%)	6(23%)	4(7%)
10 weeks or more	-*	9(11%)	5(19%)	4(7%)
11 weeks or more	-*	5(6%)	3(12%)	2(3%)
12 weeks or more	-*	3(4%)	2(8%)	1(2%)
Not determined	1(1%)	8(10%)	7(27%)[a]	1(2%)[b]

Table 1 Percentage of patients that reached a specific (or longer) steady state interval
Total study population: WCD-stop, RS, CT and PR.
Active disease: RS2 and CT .
*After reaching an interval of 8 weeks, treatment was discontinued for the WCD-stop and RS1 group, hence no values are displayed after 8 weeks.
[a]Due to a short treatment duration in the second treatment episode;
[b]Due to inconclusive activity scores.

Patients with early clinical response to omalizumab (UAS7 ≤ 6 or UCT ≥ 12 within one month of treatment) were more likely to extend the interval to a steady state interval of six weeks (or longer) and eight weeks (or longer) as compared to patients with a delayed response (87% versus 70% p=0,021, and 71% versus 51% p=0.034 respectively).

To specifically investigate the effect of interval prolongation in patients with underlying active, but well-controlled disease due to omalizumab treatment, we focussed on patients who restarted omalizumab and patients on continuous treatment (RS2 and CT group). Patients who successfully discontinued treatment (WCD-stop and RS1 group) are more likely to be in complete remission, and may therefore bias effective treatment intervals. The median steady state interval of patients with active disease was seven weeks (IQR 5-8) and analyzing the two sub-groups RS2 and CT separately; 8 weeks (IQR 7-10) and 6 weeks (IQR 5-7) ($p<0,001$) respectively. 25% of patients with an active disease were not able to extend the interval between administrations beyond four weeks. In the group of patients with active disease, 60% and 25% of patients reached a steady state interval of six and eight weeks or longer, respectively. Steady-state intervals were not associated with specific CU phenotypes (CU phenotypes depicted in table E1).

Successful implementation of tapering (by increasing treatment interval) and discontinuing omalizumab treatment in CU patients has recently been shown by several studies.[12, 14, 18, 75, 76] However, detailed data on varying omalizumab treatment intervals in patients with active CU are limited. Two previous smaller studies found that treatment intervals could be extended to at least six weeks in, respectively, 80% (n=20) [18] and 43% (n=7) [16, 17] of patients with active CSU. Uysal et al[18] also showed that 30% of patients could be treated with an 8-week interval which is comparable to our data (25%).

This is the first study with an in-depth analysis of omalizumab treatment intervals in a large population with active CU. 75% of the patients with active disease successfully extended treatment intervals between omalizumab administration beyond four weeks; 60% to six weeks or more and 25% to eight weeks or more. Our data support the possibility of extending the recommended treatment interval of four weeks while maintaining adequate disease control. Patients with an early response to treatment or with a second treatment episode (after stopping treatment due to well controlled disease) have a higher chance to successfully extent the treatment interval. Therefore, interval extension needs to be individually managed. Reduced number of drug administrations and hospital visits may subsequently lead to substantial reduction in costs and increased quality of life.

This study was approved by the Medical Ethic Review committee (METC-Utrecht-number 18-557).

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SUPPLEMENTAL TABLES AND FIGURES

Demographic	Total study population	WCD-stop	RS1	CT	PR
	n=132	n=38	n=26	n=58	n=10
Female	95 (72%)	31(82%)	16(62%)	43(74%)	5(50%)
Age at start treatment[a]	40.0 (15.3)	40.0(15.3)	45.2(16.3)	42.1(12.4)	36.2(18.3)
Disease duration[b]	3.0 (1.2-7)	2.50(1-7)	3.00(1-8)	3.20(2-7)	1.88(1-6)
Follow-up duration[c]	n.a.	14(4-21)[o]	23(15-30)	n.a.	31(22-43)
CU phenotype					
CSU-Wheals only	25(19%)	9(24%)	5(19%)	8(14%)	3(30%)
CSU-Angioedema only	9(7%)	0(0%)	3(12%)	5(9%)	1(10%)
CSU-Wheals & Angioedema	47(36%)	13(34%)	8(31%)	22(38%)	4(40%)
CSU & CindU	49(37%)	15(39%)	10(38%)	23(40%)	1(10%)
CindU only	2(2%)	1(3%)	0(0%)	0(0%)	1(10%)
Immunosuppressive while start omalizumab	57(43%)	13(34%)	15(58%)	23(40%)	6(60%)
Prednisone	41(31%)	9(24%)	12(46%)	18(31%)	2(20%)
Ciclosporin	12(9%)	3(8%)	2(8%)	5(9%)	2(20%)
Methotrexate	4(3%)	1(3%)	1(4%)	0(0%)	2(20%)
Clinical effect omalizumab					
Complete response	69(52%)	27(71%)	18(69%)	24(41%)	0(0%)
Partial response	35(27%)	8(21%)	4(15%)	23(40%)	0(0%)
Non-response	13(10%)	0(0%)	0(0%)	3(5%)	10(100%)
Missing	15(11%)	3(8%)	4(15%)	8(14%)	0(0%)
Baseline score[d*]					
UAS7	28(17-35)	26(9-34)	28(13-33)	30(23-36)	21(12-35)
UCT	5(3-8)	4(2-9)	6(5-8)	4(2-7)	5(4-8)
AAS	27(0-55)	20(0-54)	0(0-0)	38(11-61)	25(25-25)
T-end score[e**]					
UAS7	0(0-3)	0(0-0)	0(0-0)	1(0-5)	33(26-38)
UCT	16(12-16)	16(16-16)	16(15-16)	13(9-16)	5(2-9)
AAS	0(0-0)	0(0-0)	0(0-0)	0(0-8)	31(31-31)

Table E1. Demographic and clinical characteristics of patients with CU

CU = chronic urticaria, CSU = chronic spontaneous urticaria, CindU = chronic inducible urticaria. Total study population = GR-stop, GR-RS1, CT, PR and ST; GR-stop = good response stop (no restart); GR-RS1 = good response restart (first treatment episode); GR-RS2 = good response restart (second treatment episode); CT = continuous treatment; PR = treatment stopped due to poor response; Complete response was reached when UAS7 score=0 or UCT=16. Partial response was defined as improvement of disease activity by a minimal important difference (MID) of 10 UAS7 points or, if UAS7 was not available, 3 UCT points.[12, 13]

[a]mean (±SD) in years;

[b]disease duration in years before start of omalizumab, median (IQR 25-75);

[c]Median number (IQR) of months between last dose of omalizumab and data lock.

[d]before the start of omalizumab treatment, median (IQR 25-75);

Table E1 Continued

[e]at the time of the final analysis or end of treatment, median (IQR 25-75);

[o]Four patients had a post-treatment follow-up period shorter than 12-weeks.

*data available for respectively 90, 109 and 15 patients; in WCD-stop for 29, 35 and 4 patients; in RS1 for 13, 19 and 1 patients; in CT for 41, 47 and 9 patients; in PR for 7, 8 and 1 patients;

**data available for respectively 119,125 and 69 patients; in GR-stop for 37, 37 and 19 patients; in GR-RS1 for 22, 26 and 14 patients; in CT for 53, 53 and 35 patients; in PR for 7, 9 and 1 patients;

CHAPTER 6

Clinical profile of idiopathic angioedema based on severity and treatment response is independent of the presence of concomitant wheals

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ABSTRACT

Background: Idiopathic angioedema varies in disease severity and treatment response, possibly due to different pathophysiological mechanisms. The presence of wheals is an indicator for histamine mediated angioedema. Idiopathic angioedema patients are treated in accordance with CSU guidelines. Little is known about treatment effectiveness in idiopathic angioedema patients without wheals in comparison to idiopathic angioedema patients with concomitant wheals.

Objective: To describe the disease severity profile in patients with angioedema of unknown cause in relation to prophylactic treatment and the presence or absence of concomitant wheals.

Methods: In this retrospective cohort study, all records of angioedema patients visiting the outpatient clinic of the UMC Utrecht between January 2015 and March 2020 were screened. Patients with idiopathic angioedema, including those with concomitant wheals, were included. Attack frequency, patient-reported disease control and attack treatment as indicator for severity were analysed in relation to prophylactic treatment at follow-up and outcomes were compared between patients with and without concomitant subordinary wheals.

Results: 236 patients were included: 95% (139/236) with angioedema only and 41% (97/236) with angioedema and concomitant subordinary wheals. No prophylactic treatment was prescribed in 27% (64/236), with well-controlled disease in 86% (25/29) of patients. Antihistamine monotherapy was used in 59% (139/236) of patients and resulted in well-controlled disease in 68% (62/92). Add-on treatment was prescribed in 14% (33/236) of patients, omalizumab in 9% (22/236) specifically, with complete response in 38% (6/16) of patients and low attack frequency in another 18% (3/16). Difficult-to-treat disease was seen in 8% (18/236), with no response to a four-fold dose of antihistamines or omalizumab. All findings were independent from presence of concomitant wheals.

Conclusion: Angioedema is well manageable in the majority of patients without prophylactic therapy or antihistamine monotherapy, but a substantial proportion does not respond to antihistamines and/or omalizumab. Treatment response was independent of the presence or absence of concomitant wheals.

INTRODUCTION

Angioedema (AE) is characterized by acute and transient localized swelling of the subcutaneous and mucosal tissue, affecting preferentially the facial and oropharyngeal area, the extremities and genitalia. The clinical presentation, natural course, severity and treatment response differs between and within patients.[1]

The pathophysiology of angioedema is not fully understood, but generally two vasoactive peptides are thought to mediate acute AE swellings; namely, histamine and bradykinin, which both can lead to increased vascular permeability.[2] Hereditary angioedema (HAE), acquired C1 inhibitor deficiency (AAE-C1-INH) and angiotensin converting enzyme inhibitors induced angioedema (ACEI-AE) are considered as bradykinin mediated AE,[2] for which treatment is based on restoring C1-INH plasma levels and regulating the production of bradykinin.[2] Histamine mediated AE is classified as acquired AE (AAE) and is associated with chronic spontaneous urticaria (CSU).[3] An estimated 33-67% of CSU patients suffer from both (subordinary) wheals and angioedema.[4] Therefore, the presence of wheals in AE patients is currently an indicator for CSU diagnosis. Following CSU guidelines, after exclusion of C1-INH deficiency, ACEI-AE or urticarial vasculitis,[5,6] prophylactic treatment of AAE patients with or without wheals starts with antihistamine monotherapy up to a four-fold daily dose. In daily practice, these patients are classified into idiopathic histaminergic AAE (IH-AAE) and idiopathic non-histaminergic (Inh-AAE) AAE based on their clinical response to antihistamine therapy.[7] The next treatment step in antihistamine refractory patients is add-on treatment with omalizumab (anti-IgE).[3] When additional therapy with omalizumab is not effective, treatment alternatives are sparse.

Currently, little is known about the effectiveness of omalizumab in patients with idiopathic AE. The working mechanism is not fully understood[7] and data is based on case studies with, in total, only 30 individual patients, which describe complete response in 57% and partial response in 43% of idiopathic angioedema patients without wheals.[8,9] Overall, in previous studies therapeutic response to prophylactic AE treatment is based on attack frequency.[10–13] To obtain a more complete perspective on the disease severity, it is important to also take into account the patient's perspective on the disease and the required attack treatment as an indicator of the attack severity.

The primary aim of this study is to describe the profile of idiopathic AE, both histaminergic and non-histaminergic, in a large population of patients by assessing the attack frequency, patient-reported disease control and attack severity in relation to the prescribed prophylactic treatment including add-on therapy and to investigate whether there is a difference between idiopathic AE patients with or without concomitant wheals.

MATERIAL AND METHODS

Study design and population

A retrospective cohort study was performed at the University Medical Centre Utrecht (UMCU). All patients with a diagnosis related to angioedema who visited the outpatient clinic of the Dermatology/Allergology, Otorhinolaryngology, Haematology, Rheumatology and Internal Medicine departments between the 1st of January 2015 and the 1st of March 2020 were selected and screened for inclusion. Patients were included when they were diagnosed with idiopathic angioedema without (AE-only) or with concomitant subordinary wheals (AE-wheals). Patients were excluded, when the angioedema was induced by a specific trigger such as known allergy, ACE inhibitor use or when angioedema due to C1-INH deficiency (hereditary or acquired) was proven. To avoid selection bias concerning treatment response, patients without at least one follow-up visit and a follow-up duration less than 90 days were excluded. The complete selection process is presented in Supplemental Figure S1. The local medical ethics committee declared that WMO approval was not required (protocol number 20-327).

All data was extracted from the electronic patient records. Cohort characteristics and disease history (eg affected location, treatment history) were collected from the first visit. Maximum prophylactic treatment prescribed between first visit and follow-up was categorized as 'none', 'antihistamine monotherapy' and 'add-on treatment' including, montelukast, omalizumab and tranexamic acid.

After maximum prophylactic treatment was prescribed the outcome measures were collected at follow-up. Attack frequency and intensity of attack intervention were collected as indicators of AE severity. Attack frequency was categorized as: 0 attacks; < 1 per year to 1 per year; 1 per 6 months to 1 per 2 months; at least 1 per month. The intensity of attack intervention was categorized as: 1. 'not needed'; 2. 'oral rescue medication' including antihistamines, corticosteroids or tranexamic acid; 3. Parenteral rescue medication such as 'adrenalin'; 4. 'urgent care' including a visit (and treatment received) at the emergency room or hospitalization in ICU. Patient-reported disease control was categorized as 1. 'complete control' (no symptoms); 2. 'good control' (minimal symptoms; no impairment); 3. 'partial control' (improvement, but still substantial impairment); 4. 'no improvement' (substantial impairment). Data regarding attack frequency, patient-reported disease control and attack treatment was not available for all patients and availability was different for the three outcome measures.

Statistical analysis

Statistical analyses were performed by using IBM SPSS Statistics 25.0.0.2. Graphs were produced using GraphPad Prism 8.3 and Microsoft Excel. Comparison between two groups was made by t-test or Mann-Whitney U test for continuous variables that are

normally or non-normally distributed, whereas the One-way ANOVA or Kruskal Wallis were used for comparison between three groups. Comparisons of categorical variables were made by the Chi-square test in case of two groups and the Fisher-Freeman-Halton exact test was used in case of three groups.

RESULTS

In total, 236 patients (68% female; mean age 46 years) were confirmed to have idiopathic angioedema with a median follow-up time of 30 months. The majority of patients (n= 139; 59%) had idiopathic AE without wheals (AE-only) and 41% (n=97) idiopathic AE with concomitant subordinary wheals (AE-wheals). Patient characteristics are shown in table 1. AE-only patients were significantly older and suffered more often from tongue swelling and dysphonia compared to AE patients with wheals (Table 1).

	Total (n=236)	AE-only (n=139)	AE-wheals (n=97)
Female (%)	161 (68%)	93 (67%)	68 (70%)
Age (years)	46 (15-90)	**48 (16-85)***	**43 (15-90)**
Affected locations			
Facial and neck area	*209 (89%)*	*122 (88%)*	*87 (90%)*
Face	71 (30%)	45 (33%)	26 (27%)
Ear	3 (1%)	3 (2%)	0 (0%)
Eye	100 (42%)	60 (43%)	40 (41%)
Lip	148 (63%)	**74 (54%)****	**74 (76%)**
Cheek	42 (18%)	28 (20%)	14 (14%)
Neck	11 (5%)	9 (7%)	2 (2%)
Oral area, pharynx, larynx	*146 (62%)*	*90 (65%)*	*56 (58%)*
Tongue	102 (43%)	**70 (50%)*****	**32 (33%)**
Throat	76 (32%)	38 (27%)	38 (39%)
Oropharynx	11 (5%)	6 (4%)	5 (5%)
Larynx	3 (1%)	2 (1%)	1 (1%)
Peripheral	56 (24%)	30 (22%)	26 (27%)
Abdominal	10 (4%)	5 (4%)	5 (5%)
Urogenital	11 (5%)	6 (4%)	5 (5%)
Other†	8 (3%)	6 (4%)	2 (2%)
Unknown	1 (0.4%)	0 (0%)	1 (1%)

Table 1: Cohort characteristics
† Chin, nose; Significant differences between AE-only and AE-wheals patients are marked bold; p-values: * 0.046, ** <0.001, *** 0.011.

Prophylactic treatment strategy: the majority of patients respond to antihistamine monotherapy

At first presentation, 64% (n=139) of patients reported a high attack frequency of at least 1 attack per month, which was reported significantly more often in AE-wheals patients compared to AE-only patients (p=0.001, n=69; 78% and n=70; 54%, respectively).

Only 39% (n=92) of patients in our cohort used prophylactic treatment at first presentation. This was increased during follow-up to 73% (n=172) of patients. The maximum prescribed prophylactic treatment (required for disease control) during follow-up could be differentiated into three strategies: 27% (n=64) of patients with no prophylactic treatment; 59% (n=139) with antihistamine monotherapy; and 14% (n=33) with add-on treatment. AE-wheals patients received prophylactic treatment with antihistamines or add-on treatment more often compared to AE-only patients (Table 2).

Maximum prophylactic treatment	Total (n=236)	AE-only (n=139)	AE-wheals (n=97)	p-value
None	64 (27%)	54 (39%)	10 (10%)	<0.001
Antihistamine monotherapy	139 (59%)	73 (53%)	66 (68%)	0.017
1-2 fold dose	38 (16%)	22 (16%)	16 (17%)	
3- >4 fold dose	101 (43%)	51 (37%)	50 (51%)	
Add-on treatment	33 (14%)	12 (9%)	21 (22%)	0.005
Montelukast	6 (3%)	1 (1%)	5 (5%)	
Omalizumab	22 (9%)	9 (6%)	13 (13%)	
Tranexamic acid	5 (2%)	2 (1%)	3 (3%)	

Table 2: Maximum prescribed prophylactic treatment during follow-up
Maximum prophylactic treatment at follow-up was defined as the maximum prophylactic treatment prescribed between the first visit and the last visit before datalock.

When analysing attack frequency at follow-up, patients without prophylactic treatment reported no attacks in 86% (n=25, out of 29 with available data). Only 14% (n=4) of patients reported AE attacks at follow-up (Figure 1A; Table S1). Of these 4 patients, 1 used oral rescue medication, 2 patients sought urgent care and 1 did not report having used any rescue medication during follow-up. Studying patient-reported disease control at the end of follow-up: 87% (n=26, out of 30 with available data) of patients without prophylactic treatment experienced complete or good control of disease (Figure 1B; Table S1).

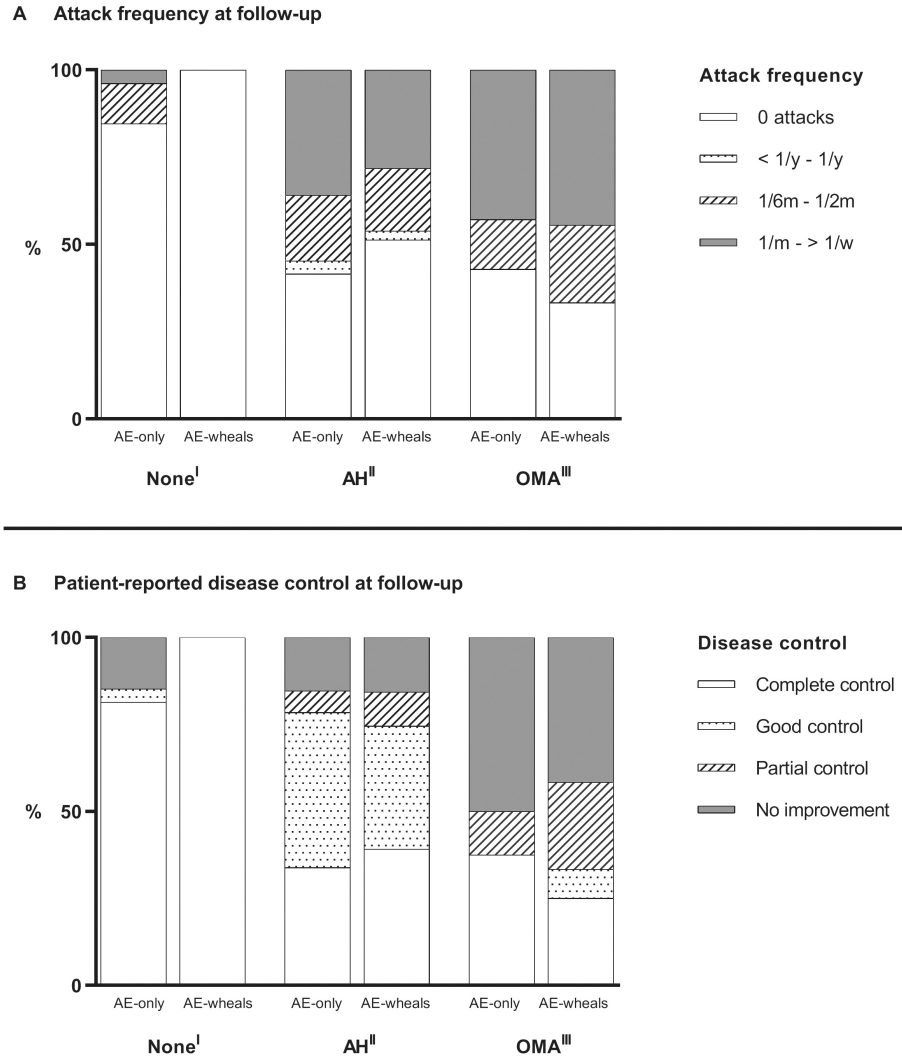

Figure 1: Attack frequency (A) and patient-reported disease control (B) per subgroup

A Attack frequency at follow-up per AE subgroup and maximum prescribed prophylactic therapy. Subgroups are displayed as bars, grouped per prophylactic treatment strategy. The shade represents the attack frequency category. N=137 as for 99 patients information on attack frequency was not available. † Angioedema patients without wheals; ¥ Angioedema patients with wheals; I none (n=29); II antihistamines (n=92); III omalizumab (n=16).

B Patient-reported disease control at follow-up per AE subgroup and maximum prescribed prophylactic therapy. Subgroups are displayed as bars, grouped per prophylactic treatment strategy. The shade represents the patient-reported disease control category. N=166 as for 70 patients information on disease control was not available. † Angioedema patients without wheals; ¥ Angioedema patients with wheals; I none (n=30); II antihistamines (n=116); III omalizumab (n=20).

Patients with antihistamine monotherapy (in any dose) reported no attacks at follow-up in a considerably lower percentage: 46% (n=42, out of 92 with available data). Low attack frequency (< 1 per year to 1 per 2 months) was reported by 22% (n=20) of patients and even high attack frequency (at least 1 attack per month) was reported by 33% (n=30) of patients (Figure 1A;

Table S1), indicating relatively poor disease control in the antihistamine monotherapy group at follow-up based on attack frequency. Looking at patient-reported disease control at the end of follow-up, 77% (n=89, out of 116 with available data) of patients with antihistamine monotherapy reported complete or good control of disease (Figure 1B; Table S1).

Studying the effectiveness of a four-fold daily dose of antihistamines, specifically, this was prescribed in 41% (n=96) of patients during follow-up. Complete remission (0 attacks) or a low attack frequency (< 1 per year to 1 per 2 months) was seen in 64 % (40% and 24% respectively; n=43 out of 68 with available data) at follow-up. 36% (n=25) patients continued to suffer from high attack frequency with at least one attack per month. Response to four-fold daily dose antihistamines was independent of the presence of wheals.

Add-on treatment was prescribed to 14% (n=33) of patients during follow-up (Table 2), of which omalizumab was most often used; 9% (n=22) of cases. Further analysis of therapeutic response was only performed for omalizumab patients. Data about specific attack frequency at follow-up was available for 16 patients using omalizumab; 38% (n=6) of patients experienced no attacks and 18% (n=3) reported low attack frequency (1 per 6 months to 1 per 2 months), but 44% (n=7) at least one attack per month (Figure 1A; Table S1).

However, based on patient-reported disease control at the end of follow-up, 35% (n=7 out of 22) of all patients treated with omalizumab reported complete or good disease control after addition of omalizumab to antihistamine treatment, while 65% (n=13) of patients had partial or no disease control, suggesting high burden, also in patients with relatively low attack frequency.

Acute attack treatment use was largely reduced after initiation of prophylactic treatment

To obtain an indication of the severity of attacks, acute attack treatment was analysed per prophylactic treatment strategy: no prophylactic treatment, antihistamine monotherapy, omalizumab. Acute attack treatment use and emergency intervention was decreased during follow-up compared to at first presentation (n=59, 42% versus n=182, 80%). At follow-up, after maximum prophylactic treatment prescription, use of urgent care was seen in 10% (n=3), 5% (n=5) and 25% (n=5) of patients with no prophylactic treatment, antihistamine monotherapy or omalizumab respectively (supplemental figure S2, Table S1).

No difference in response to prophylactic treatment between AE-only and AE-wheals patients

To identify differences between AE-only and AE-wheals patients, prophylactic treatment strategies and their treatment response upon these strategies were compared between the two groups. At first presentation, significantly more AE-wheals patients (n=52, 54%) used prophylactic treatment compared to AE-only patients (n=40, 29%, p<0.001). Similarly, during follow-up prophylactic treatment was prescribed more often (90%)

to AE-wheals patients compared to AE-only patients (61%, p<0.001) (table 2). This corresponds to a significantly higher attack frequency (at least 1 per month) in AE-wheals patients (n=69, 71%) compared to AE-only patients (n=70, 50%) at first visit, suggesting this difference is related to disease severity, but not disease entity.

Despite the higher use of prophylactic treatment in AE-wheals patients, at follow-up no difference in attack frequencies, patient-reported disease control and acute attack treatment were found compared to AE-wheals patients. Hence, therapeutic response to antihistamines or add-on treatment with omalizumab between AE-only and AE-wheals patients during follow-up did not differ.

DISCUSSION

To our knowledge, this is the first study focusing specifically on the disease severity profile related to prophylactic treatment response in a large population of idiopathic AE patients and additionally differentiating between AE patients with and without concomitant subordinary wheals. The percentages of maximum prescribed prophylactic treatment during follow-up were distributed as (1) 27% with no prophylactic treatment; (2) 59% antihistamine monotherapy and (3) 14% of patients with add-on treatment, of which 9% was with omalizumab. Well-controlled disease was seen in the majority of patients with no prophylactic treatment or antihistamine monotherapy (86% and 68% respectively) showing no attacks or low attack frequency only. Of patients with, specifically, a four-fold dose of antihistamines, 64% showed no or minimal symptoms (complete or good response). A relatively small, but substantial proportion required add-on treatment with omalizumab (9%), of which more than half of patients were well-controlled with no attacks or low attack frequency; but still 44% suffered from a high attack frequency during follow-up. Notably, we found no significant difference in treatment response to antihistamines or omalizumab between patients with angioedema with and without wheals.

In order to obtain a more comprehensive impression of disease severity, we analysed additional outcomes such as patient-reported disease control and acute attack treatment. Resembling attack frequency, the majority of patients without prophylactic treatment or antihistamine monotherapy reported no or minimal symptoms (complete or good control response) (87% and 77% respectively). Furthermore, we found a decrease in use of acute attack treatment and intervention after maximum prophylactic treatment prescription at follow-up, suggesting not only lower attack frequency, but also milder attacks due to maximum prophylactic treatment for each prophylactic treatment strategy. No other studies have looked into acute attack treatment in relation to prophylactic treatment.

No prophylactic treatment was used in a substantial part of our population (27%), of which the majority showed no attack during follow-up, illustrating that the natural course of the disease can be mild and self-limiting. This was also supported by our finding that 81% of this group did not use any attack treatment. In our literature review, we did not find any data allowing comparison of the disease in AE patients without prophylactic treatment with other populations.

Antihistamine monotherapy, used by 59% of our population, resulted in complete response and low attack frequency in 46% and 22%, respectively. In preceding studies, the effect of antihistamines in AE patients was analysed with regard to the current classification of AAE, which defines patients as IH-AAE or InH-AAE based on the response to antihistamine therapy.[2,3] The effect of antihistamines on AE was previously studied mainly in angioedema patients without wheals.[10–13] These studies found similar complete response rates at follow-up after antihistamine monotherapy (in variable dosage) ranging from 56 to 72%[10,11] and improvement of attack frequency in 86-91%.[12,13] Notably, in our study the presence of wheals did not influence antihistamine therapy outcome, resulting in an antihistamine response rate of 68% in AE patients with and without wheals that is comparable with the response rates in AE patients without wheals as shown in these previous studies.

9% of our patients received omalizumab, which led to complete response in 38% and low attack frequency in 18% of patients, which was comparable for AE patients with and without wheals. Previous studies investigated the effect of omalizumab for CSU patients and showed well-controlled disease in 52-63%[14,15] and complete response in 34-40% of CSU patients,[15] which are higher rates compared to our results. Data regarding therapeutic response to omalizumab in AE patients without wheals is limited to 10 case reports with a total of 30 patients. These reports showed response in all cases with complete response in 57% (n=17) and partial or good response in 43% (n=13) of patients.[8,9] However, due to the sensitivity of case reports to publication bias, effectiveness of omalizumab in angioedema patients in current literature is probably overestimated. Therefore, we expect real-world effectiveness of omalizumab for patients with idiopathic AE to be lower and correspond more to the percentage found in our study, which needs to be proven in prospective studies.

In conformance with CSU guidelines,[2,3] in daily practice, patients with idiopathic AE are treated with omalizumab in cases of insufficient response to a four-fold dose of antihistamines. Given the data of this study, we can extrapolate that approximately 92% of the AE population is well-controlled without prophylactic treatment, antihistamines or add-on treatment and that 8% remain difficult to treat due to unresponsiveness to these therapeutic options and absence of other available treatment options. A notable finding in our study was that the response to antihistamines and omalizumab was irrespective of the presence of wheals. This implies that the presence of wheals is not

indicative of an underlying histamine mediated mechanism and that other common pathophysiologic mechanisms, such as bradykinin, might play a role. This concept was recently suggested in a study demonstrating elevated plasma Csk-homologous kinase levels in CSU patients compared to healthy controls.[16] This finding, however, could not be related to clinical response to antihistamines. Nonetheless, bradykinin might contribute to the pathophysiologic mechanism of AE and wheals, since previous research has shown that the bradykinin-forming contact system is involved in mast cell mediated reactions and processes.[17] This may explain limited treatment response for both symptoms in patients with a certain phenotype. Further research is needed to unravel the specific pathophysiologic mechanism in patients suffering from angioedema and/or wheals not responding to antihistamines or anti-IgE therapy. Such research will enable better treatment strategies to be explored.

Although we found no difference in treatment response between AE patients with and without wheals, AE patients without wheals were older (48 versus 43 years) and reported more often a swelling of the tongue compared to AE patients with wheals (50% versus 33%). This is also described in a recent prospective study, which found significantly more frequent tongue swellings in AE patients without wheals compared to AE patients with wheals (59% vs. 29%).[18] Tongue swelling is frequently seen in patients with IH-AAE, C1-INH deficiency and ACEI-AE.[5] However, it is questionable whether this specific symptom can be explained by a specific pathophysiologic mechanism (e.g. histaminergic or bradykinin mediated AE) in patients with or without wheals. Yet, we did not find any differences in response to antihistamines based on attack frequency and patient-reported disease control between patients with and without tongue swelling.

An important advantage of this study is the large cohort of idiopathic AE patients with a defined minimum follow-up period of 90 days. However, several limitations need to be mentioned: its retrospective design, resulting in missing values and variations in follow-up duration, and the lack of validated patient outcome scores angioedema activity score (AAS) and urticaria control test (UCT) which were only available in a proportion of patients from recent years, resulting in more subjective outcomes.

In conclusion, we describe an idiopathic AE population profile showing a majority of patients with well-controlled disease requiring no prophylactic treatment or antihistamine monotherapy only. A minority requires add-on treatment with insufficient response in a substantial proportion. AE severity profile and treatment response was independent from presence of wheals.

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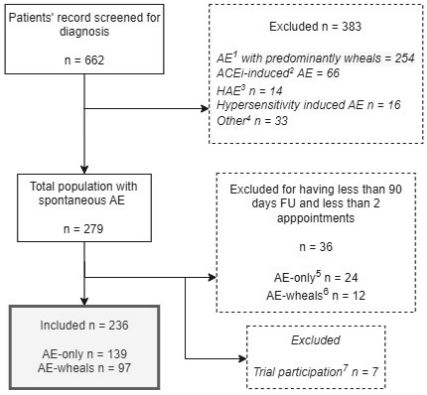
SUPPLEMENTAL TABLES AND FIGURES

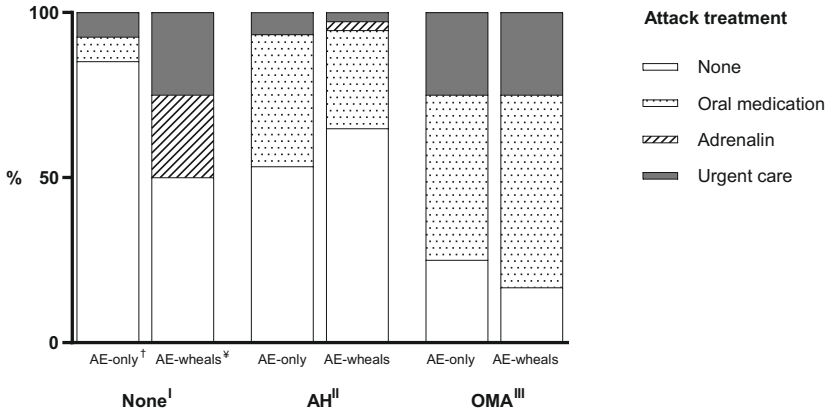
Prophylactic treatment[I]†	None[I]		AH[II]		OMA[III]	
	AE-only[£]	AE-wheals[¥]	AE-only	AE-wheals	AE-only	AE-wheals
Attack frequency (n)‡	**26**	**3**	**53**	**39**	**7**	**9**
0 attacks	22 (84%)	3 (100%)	22 (41%)	20 (51%)	3 (43%)	3 (33%)
< 1/ year to 1/ year	0 (0%)	0 (0%)	2 (4%)	1 (3%)	0 (0%)	0 (0%)
1/ 6m – 1/ 2m	3 (12%)	0 (0%)	10 (19%)	7 (18%)	1 (14%)	2 (22%)
≥ 1/ m	1 (4%)	0 (0%)	19 (36%)	11 (28%)	3 (43%)	4 (45%)
Patient-reported disease control (n)‡	**27**	**3**	**65**	**51**	**46**	**39**
Complete control	22 (82%)	3 (100%)	22 (34%)	20 (39%)	3 (38%)	3 (25%)
Good control	1 (4%)	0 (0%)	29 (45%)	18 (35%)	0 (0%)	1 (8%)
Partial control	0 (0%)	0 (0%)	4 (6%)	5 (10%)	1 (12%)	3 (25%)
No improvement	4 (14%)	0 (0%)	10 (15%)	8 (16%)	4 (50%)	5 (42%)
Attack treatment (n)‡	**27**	**4**	**45**	**37**	**8**	**12**
None	23 (86%)	2 (50%)	24 (53%)	24 (65%)	2 (25%)	2 (17%)
Oral medication	2 (7%)	0 (0%)	18 (40%)	11 (29%)	4 (50%)	7 (58%)
Adrenalin	0 (0%)	1 (25%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)
Urgent care	2 (7%)	1 (25%)	3 (7%)	1 (3%)	2 (25%)	3 (25%)

Supplemental table S1: Prophylactic treatment response at follow-up
Based on attack frequency, patient-reported disease control and acute attack treatment, sorted by subgroup. † Maximum prescribed prophylactic treatment during follow-up, ‡ The number of patients of whom this data was available, £ Angioedema patients without wheals, ¥ Angioedema patients with wheals, I No prophylactic treatment during follow-up, II Antihistamine monotherapy in various dosage, III Omalizumab. Statistical analysis was performed using the Fisher's exact test.



Supplemental Figure S1: Flowchart: patient screening and selection

1. Angioedema; **2.** ACE inhibitor use: still using (n=2), stopped > 1 year (n=18), stopped < 6 months (n=20), stopped > 6 months (n=7), stopped during follow up (n=16), unknown when stopped (n=3); **3**. Hereditary angioedema; **4**. Edema of other etiology; **5**. Angioedema without wheals; **6**. Angioedema with concomitant subordinary wheals.**7.** AE-only patients who participated in clinical trials and were therefore treated with different protocols.

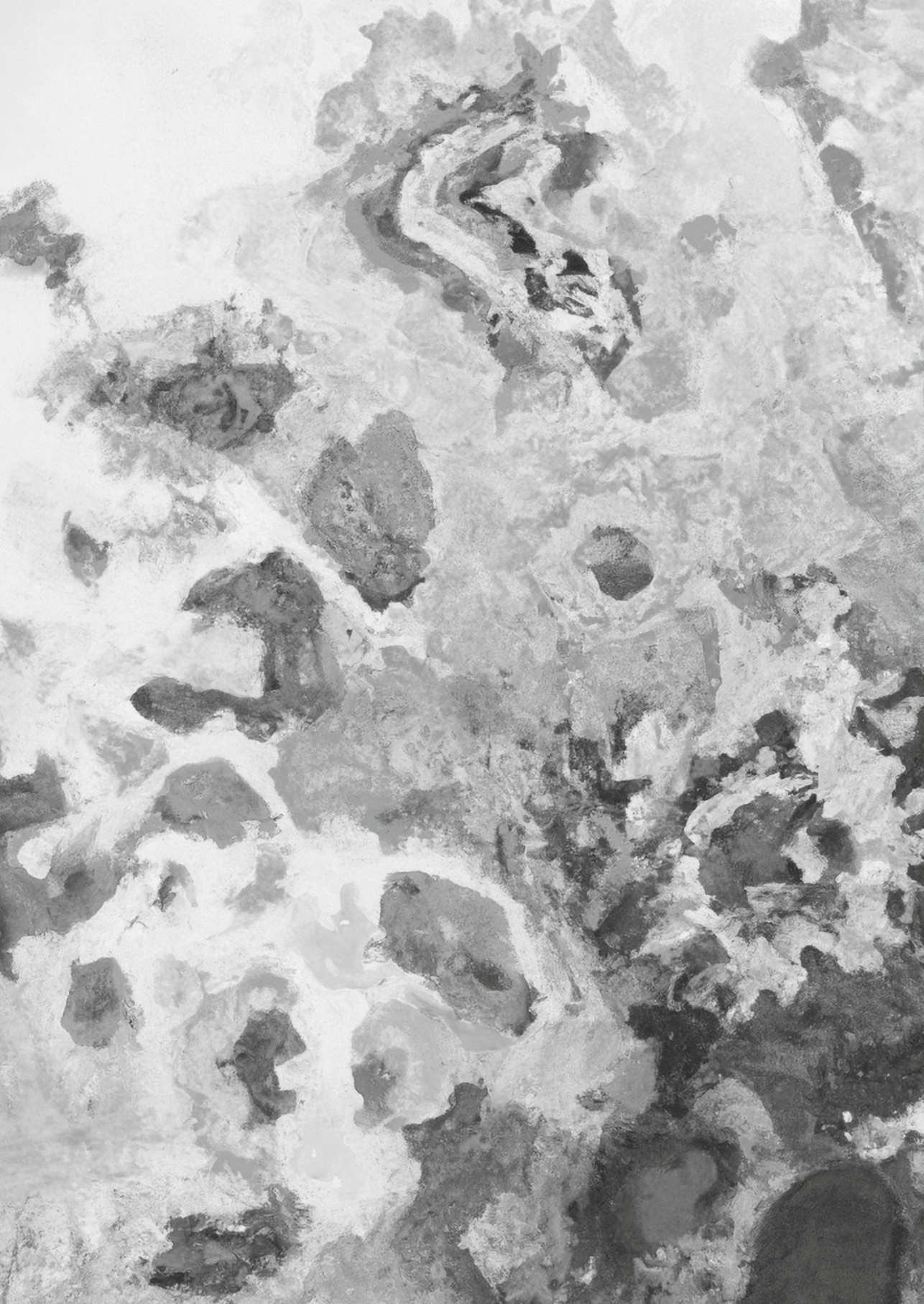


Supplemental Figure S2: Used attack treatment after prescribing maximum prophylactic treatment

Attack treatment at follow-up per AE subgroup and maximum prescribed prophylactic therapy. Subgroups are displayed as bars, grouped per prophylactic treatment strategy. The shade represents the attack treatment category. N=133 as for 103 patients information on attack frequency was not available. † Angioedema patients without wheals; ¥ Angioedema patients with wheals; I none (n=31); II antihistamines (n=82); III omalizumab (n=20).

PART II

Exploring the pathomechanistic pathways involved in CU and working mechanism of omalizumab and recombinant human C1-esterase inhibitor (rhC1-INH).



CHAPTER 7

Systemic and Local Evidence for Complement involvement in Chronic Spontaneous Urticaria.

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ABSTRACT

Background: The pathogenesis of chronic spontaneous urticaria (CSU), including the mechanism of action of omalizumab, remain unclear. We hypothesized complement system involvement given the often fast clinical response induced by treatment, including omalizumab. Therefore, we assessed the role of various complement factors surrounding omalizumab treatment.

Methods: 30 CSU patients (median age 42 [range 21-70]; 73 % female) with a median once daily Urticaria Activity Score over 7 days (UAS7) score at baseline of 31.5 points were enrolled. Treatment consisted of 6 administrations of 300 mg omalizumab every four weeks succeeded by a follow-up period of 12 weeks. Four punch skin biopsies were taken per patient; at baseline from lesional skin, at baseline from non-lesional skin, and after one and seven days from formerly lesional skin. Complement activity, including C1q, C3, C3bc/C3, C4, C4bc/C4, C5a and Membrane Attack Complex (MAC) in peripheral blood were analysed and complement activation in the skin was determined by the analysis of C4d deposition. Results were related to the clinical response to omalizumab.

Results: Fifteen patients showed a UAS7 score of six or lower (median 0) at week 24, fifteen patients did not (median 16). Lesional skin biopsies at baseline revealed complement deposition (C4d) in blood vessels in the papillary dermis of 53% (16/30) of the patients, which suggests involvement of immune complexes in the pathogenesis of urticaria. Moreover, indication of increased complement activation in CSU was substantiated by increased C5a levels in peripheral blood compared to healthy controls ($p=0.010$). The clinical effect of omalizumab could not be linked to the variation of complement components.

Conclusions: Both C4d deposition in lesional skin and elevated C5a levels in peripheral blood indicate the involvement of complement activation in the pathogenesis of CSU. No correlation was found between omalizumab and activation of complement indicative of independent processes in the immunopathogenesis of CSU.

INTRODUCTION

Chronic spontaneous urticaria manifests as an burdensome skin disease with sudden onset, sometimes severe itching and wheals, that lasts for at least 6 weeks. Prevalence is estimated to be up to 1% at any time, with disease duration ranging from 1 to 5 years or even longer in more severe cases.[77] Omalizumab has a reported clinical response of over 50% within the first two days of treatment in CSU patients.[78] Depletion of free IgE by omalizumab leads to down-regulation of the FcεRI on mast cells[79] and basophils[80] in patients with allergic disease.[81] However, this down-regulation alone cannot explain the fast clinical response to omalizumab. Hence, alternative mechanisms must be contributing to the rapid clinical efficacy of omalizumab.

Sera from patients with urticaria can induce degranulation of basophils, a process in which the presence of intact complement and patient IgG containing specific antibodies against IgE or the high affinity IgE receptor is essential.[82] By binding to IgE or FcεRI on mast cells, complement via the classical pathway can be activated and lead to the generation of C5a and C5b-9.[83] C5a can subsequently bind to the complement C5a receptor on mast cells and cause degranulation. Cutaneous mast cells express the complement C5a receptor whereas mucosal mast cells do not. Additionally, the complement system is known for its rapid response upon activation. This may explain how IgG anti-FcεRI autoantibodies in combination with complement in patients with CSU can cause fast clinical symptoms which are limited to the skin and not mucosal tissue.[84]

It has been reported that C1q, C2, C3, C4, and C5 levels in peripheral blood are within normal limits in chronic urticaria but no data has been published regarding complement degradation/activation products in peripheral blood.[85-87] The effect of omalizumab treatment on peripheral blood complement levels in CSU patients has also never been studied.

Furthermore, it is unknown whether complement activation occurs in the skin of patients with CSU. Complement activation in tissue can be evaluated by determination of C4d deposition: a well-studied marker and a characteristic feature of complement activation, which is, for instance, also included in the BANFF criteria for humoral rejection after kidney transplantation.[88] In this study, we investigated the role of the complement system and the effects of omalizumab treatment in CSU patients using the C4d marker in skin and peripheral blood samples. Additionally, we hypothesize that the efficacy of omalizumab in CSU may in part be accompanied by reduction of complement mediated inflammation.

METHODS

Design and population

This monocenter exploratory prospective cohort study was performed in the University

Medical Center Utrecht, the Netherlands from 2015 until 2017. Inclusion criteria were adult CSU patients with a significant disease activity defined as a once daily Urticaria Activity Score over 7 days (UAS7) ≥ 16 and a UAS7 ≥ 4 on the day of the first omalizumab administration despite treatment with antihistamines up to four times the daily dose. Exclusion criteria were based on one of the pivotal randomized controlled trials (RCTs)[89] and included a clearly defined underlying etiology for chronic urticaria (e.g. chronic inducible urticaria [CINDU]), a history of malignany, known hypersensitivity to omalizumab, and pregnancy. Routine administration of immunosuppressants including prednisolone and ciclosporin[70] were discontinued with washout periods of 3 months prior to treatment with omalizumab. If prednisolone was used as rescue medication, a washout period of 2 weeks was maintained before the start of the study. After a screening period of up to 2 weeks, eligible patients started a six-month treatment period, followed by a follow-up period of 3 months. The latter could be shortened upon patient-request if the UAS7 was projected to reach a score of 16 or higher. All patients provided written informed consent, and the study was approved by the local ethics committee (protocol number 15-167).

Omalizumab and concomitant medication

All patients received 6 doses of 300 mg omalizumab every 4 weeks with follow-up starting at week 25, four weeks after the last dose. Leukotriene receptor antagonists (LTRA) or H2 blockers for indications other than CSU were permitted to be continued during the study. Patients were allowed to use H1-antihistamines up to a maximum of 4 doses per day as rescue medication in addition to their concomitant medication, as well as prednisolone up to 30 mg. Due to worsening of the disease, 11 patients, of which 6 (55%) were presented as responders, restarted omalizumab treatment during follow-up. Data of subjects who restarted omalizumab during the follow-up period were removed from data analysis from that consecutive time-point. In absolute numbers, the number of patients who restarted omalizumab were: 1 in week 25, 2 in week 26, 3 in week 28, 4 in week 29, 9 in week 30, and 11 in week 32.

Assessments in blood samples

Blood samples were collected at the following time-points: at baseline, after 1, 2, 6, and 24 hours, after 1 and 2 weeks, and 4 weeks after the first administration of omalizumab. Subsequently, blood was collected prior to each subsequent dose. Lastly, a venipuncture was performed at the last follow-up visit. For measurement of complement activation, EDTA plasma, serum and gel separated serum were used. EDTA blood and gel separated serum were put on ice immediately after venipunctures. All serum samples were allowed

to coagulate for 60 minutes. Serum and plasma were obtained by centrifugation and stored at -80 ºC.

Measurements complement

Complement levels of C3 and C4 were determined in serum by an immunonephelometric method on a SPA+ turbidimeter, C5b-9 membrane attack complex (MAC) formation via the classical complement activation route was measured in gel separated serum using a commercially available enzyme-linked immunosorbent assay (ELISA, EuroDiagnostica, Sweden) according to the manufacturer's recommendations, and C5a was determined in EDTA plasma via Luminex xMAP technology (Luminex Corporation, United states). Additionally, C1q in serum, and C3bc and C4bc in EDTA plasma were determined as previously described.[90]

Skin biopsies

A total of four 3mm punch skin biopsies were taken per patient; 1) at baseline from lesional skin, 2) at baseline from non-lesional skin, and after one (3) and seven days (4) from formerly lesional skin. Skin sections were formalin-fixed, paraffin-embedded, and stained by immunohistochemistry with specific antibodies allowing characterization of inflammation (HE-staining), CD3 (DAKO), CD4 (Cellmarque), CD8 (DAKO), CD20 (Roche), CD68 (Leica), CD138 (Serotec), and 2D7-antibody (Hycult)). All characteristics were visually examined and judged on a 0 to 3 semi-quantitative scale[91, 92] as 'not elevated' (0) or a mild, moderate or severe increase (1-3), on original magnification 400x. Complement activation in the skin was evaluated by determination of C4d deposition (polyclonal rabbit anti-C4d staining, ALPCO, Salem, NH, USA), and was graded from 0 (negative) to 3 (bright signal or fully surrounding blood vessel walls) (Figure 1). As previously described, the original magnification was 400x.[92]

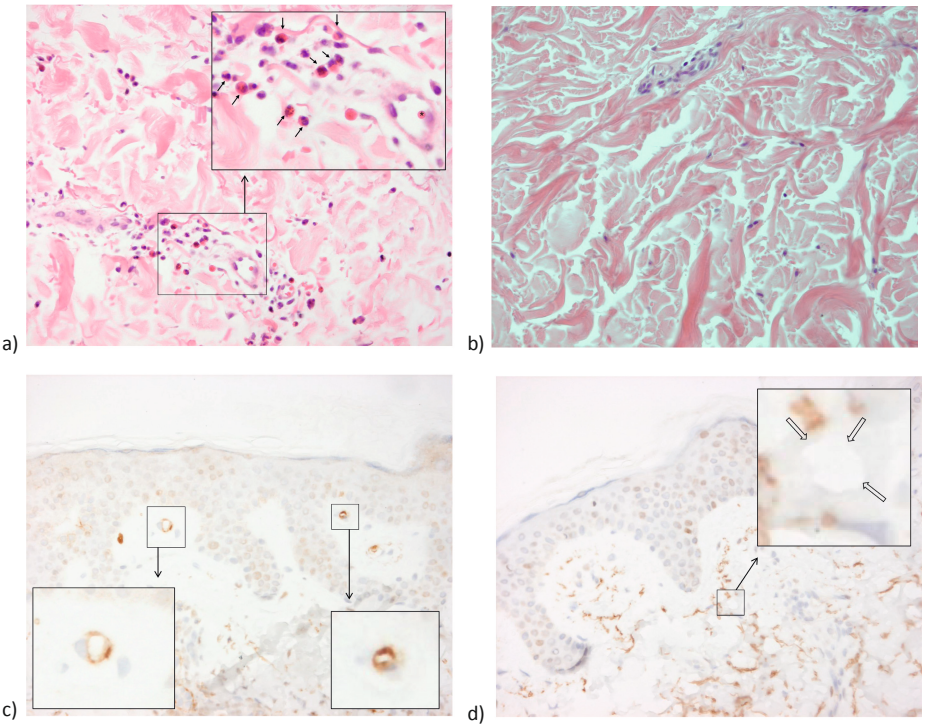

Figure 1: Inflammation and C4d deposition in skin
a) HE staining with evident edema, perivascular infiltration, limited interstitial infiltration, and many eosinophils. Arrows indicate eosinophils, *: erythrocyte within blood vessel. b) HE staining with very limited edema, no infiltration, and absence of granulocytes. c) C4d staining with evident c4d deposition fully surrounding vessel walls. d) C4d staining, open arrows indicate blood vessels with absence of C4d

Clinical assessments and patient-reported outcomes

Disease activity was measured throughout the study by using the UAS7.[93] Missing daily scores of the weekly disease activity scores after treatment was started were complemented by Last Observation Carried Forward method (LOCF) up to a maximum of three days. Missing follow-up scores were supplemented with data from each patient's clinical record following clinical visits if available. Weekly scores which could not be complemented with earlier mentioned methods, were marked as missing and were not included in patient reported outcome results. Disease control was measured at baseline, four weeks after each administration, and at the last follow-up visit by using the urticaria control test (UCT).[72]

Treatment response has been defined as a UAS ≤ 6 at week 24 of treatment.

Statistical analysis

Changes in inflammatory parameters in the skin were related to changes in levels of circulating complement components, by using Spearman Rank correlation. Inflammatory

characteristic after treatment were compared to baseline, and/or to the previous measurement, using Wilcoxon matched pairs signed rank tests, or paired samples T-test as appropriate. C3bc and C4bc activation ratios were determined by dividing the level of circulating C3bc or C4bc by the amount of C3 or C4 respectively and multiplying the quotient by 100 to determine the percentage, as previously described.[90] Study population baseline scores for C5a were compared with normal values of C5a (based on results in a pool of 43 healthy volunteers) using the Mann Whitney U test. For all statistical tests, a p-value of 0.05 or lower was considered significant.

Descriptive analyses were carried out for all clinical efficacy outcomes. For each protein, Spearman's correlations were calculated between the difference in complement level from baseline after 1 hour (C5a: after 2 hours) and the difference in UAS7 score from baseline after 1 week. Additionally, the change

from baseline in peripheral blood complement components was related to the change from baseline in UAS7, also by using Spearman Rank correlation or Pearson correlation if appropriate. Statistical analysis was performed using IBM SPSS Statistics version 21 or GraphPad Prism version 7.02, graphs were prepared using Microsoft Visio 2010 or GraphPad Prism version 7.02.

RESULTS

Population

In this study, thirty patients (median age 42 [range 21-70]; 73 % female) with a median UAS7 score at baseline of 31.5 points were enrolled. 12 of the 30 patients (40%) reported concomitant CindU complaints while 24 of the 30 patients (80%) reported an history of angioedema attacks. The median disease duration was 2.7 years (range 0.6 – 29). Detailed clinical charecteristics can be found elswhere[80] Overall, patient characteristics corresponded with the CSU population in our clinic and current literature.[9] Fifteen patients (50 %) showed a UAS7 score of six or lower (median 0) at week 24 (four weeks after the last omalizumab administration) and were defined as responders. When analysing response by the use of the minimal important difference (MID) of 10 UAS7 points, 23 patients (76,6%) were responder at week 24, which included nine complete responders (UAS7 =0) , which is fairly similar to daily practice data. Four of the 30 patients (1 responder, 3 non-responders) reported prednisolone use at some time-points during the study period.

Inflammation and complement activation in lesional and non-lesional skin

Quantification of histological alteration found in skin biopsies of patients compared to healthy controls is presented in table 1. Histological analysis demonstrated no significant differences between lesional and non-lesional biopsies at baseline or follow-up with regard to oedema and cellular infiltration. Higher amounts of C4d deposition

were significantly more frequently found in lesional skin compared to non-lesional skin (p=0.033) (table 1). In the total 60 baseline skin biopsies, there was a significant correlation between C4d deposition and eosinophils scores (Spearman's ρ 0.358, p=0.005).

Frequency of dermal changes	Lesional baseline				Non-lesional baseline				Lesional day one				Lesional day seven			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Edema																
Superficial	14	13	2	0	22	7	1	0	23	7	0	0	25	5	0	0
Deep dermis	16	9	4	0	20	9	1	0	20	8	2	0	23	7	0	0
Perivascular infiltration																
Superficial	7	19	3	1	9	21	0	0	13	15	2	0	13	15	2	0
Deep dermis	25	3	2	0	29	1	0	0	29	1	0	0	28	1	1	0
Interstitial infiltration																
Superficial	20	7	3	0	25	5	0	0	28	2	0	0	27	3	0	0
Deep dermis	19	6	3	2	27	3	0	0	26	2	1	1	27	3	0	0
T-cells:																
CD3	4	22	3	0	5	23	2	0	5	21	4	0	7	19	4	0
CD4	3	19	8	0	3	16	11	0	4	16	9	0	5	16	9	0
CD8	16	14	0	0	15	15	0	0	15	14	0	0	17	13	0	0
B-cells:																
CD20	27	3	0	0	29	1	0	0	30	0	0	0	29	1	0	0
Plasma cells	27	1	0	0	28	1	0	0	29	1	0	0	28	1	0	0
Granulocytes																
Neutrophils	14	7	7	1	26	3	1	0	24	4	1	1	27	3	0	0
Eosinophils	17	5	5	3	26	2	0	2	24	5	0	1	30	0	0	0
Basophils	24	3	2	1	27	1	1	1	27	2	1	0	29	1	0	0
Mast cells	23	6	1	0	26	4	0	0	22	7	1	0	24	6	0	0
Histiocytes	5	18	7	0	11	19	0	0	11	15	3	1	16	12	2	0
C4d deposition	13	5	4	7	17	8	4	1	19	8	1	2	19	7	3	1

Table 1: Inflammation and complement deposition in skin
Note: Score 0, not elevated; 1–3, mild, moderate, or severe increase compared to healthy skin. It was not possible to make a reliable assessment of all items in seven of 120 biopsies, therefore not all characteristics add up to 30 patients.

Complement activation in peripheral blood

Table 2 shows that in a large portion of patients, peripheral blood levels of all complement components

Protein	Normal value	Baseline measurements Reduced Elevated n (%) n (%)		Total measurements Reduced Elevated # (%) # (%)	
C1q	81 – 128 IU/mL	3/30 (10)	8/30 (27)	32/352 (9)	61/352 (17)
C3	0.9 - 1.8 g/L	1/30 (3)	0	21/352 (6)	0
C4	0.1 - 0.47 g/L	1/30 (3)	0	12/352 (3)	0
C5a	<13605 pg/mL	n.a.	2/30 (7)	n.a.	9/250 (4)
MAC	69 – 129%	5/30 (17)	1/30 (3)	21/238 (9)	5/238 (2)

Table 2: Peripheral blood complement component levels
Aberrant complement measurements for the number of patients (n) are shown at baseline and for all cumulative measurements for the following timepoints. Number of missing values C1q:8, C3:8, C4:8, C5a:8, MAC:5. MAC: C5b-9 membrane attack complex formation. 'Reduced' and 'Elevated' indicate values below lower limit of normal, or above upper limit of normal.

investigated were within normal ranges throughout the study. Most investigated complement component levels in peripheral blood were within normal ranges at both baseline and throughout the study. However, as shown in Figure 2, C5a levels at baseline (median 1847,6 pg/mL) were increased compared to healthy controls ((median 959.2 pg/mL); (p=0.010)). No disproportion was seen for aberrant values since they were either too high or too low in an equal proportion of patients. For example, C1q levels at baseline were reduced in 3 patients (10%) and elevated in 8 (27%), and throughout the study 32 (8%) C1q measurements were reduced and 61 were elevated (16%). Additionally, no correlations were found between complement component levels in peripheral blood and C4d deposition in skin.

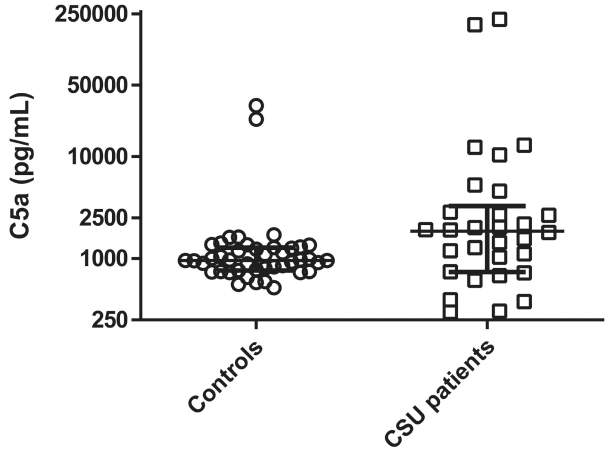

Figure 2: C5a levels at baseline of healthy controls compared to CSU patients. Data are shown as median C5a levels (IQR).

Peripheral blood complement component measurements within two hours after omalizumab injection were disregarded, since a short-term decrease of less than 10% in complement components are a known phenomenon after biological treatment.[94] No

differences in complement levels were found after two hours for all other time points compared to baseline. When comparing non-responders and responders, no statistical difference could be found in the complement component levels and their degradation products. No statistical significant correlations between difference in disease activity after 1 week and difference in peripheral blood complement levels 1 hour after the first administration were found (see Additional table 1). Additionally, no correlations were found between UAS7 after one week and both complement levels and difference in complement levels after one hour.

DISCUSSION

In this study, we investigated the role of the complement system in skin and peripheral blood in 30 patients with chronic spontaneous urticaria treated with omalizumab. Treatment with omalizumab resulted in a strong improvement in median disease activity, which was in line with previous studies[9].

This is the first study to demonstrate activation of the complement system in the both peripheral blood and skin of CSU patients. At baseline, we found complement C4d deposition in the lesional skin in 53% of the patients, and significantly higher peripheral blood C5a levels in CSU patients compared to healthy individuals, indicating complement activation in a significant percentage of CSU patients. Within the first hour after first omalizumab administration, different complement components in the peripheral blood decreased irrespective of disease activity or treatment response, indicating that omalizumab administration leads to consumption of complement components. No relation was found between complement components investigated and disease activity (UAS7) scores prior to or after treatment with omalizumab, indicating that the clinical responses induced by omalizumab are not related to short- or long-term changes in circulating levels of complement components and their degradation products.

To our knowledge, it is a novel finding that C4d is present in small blood vessel walls within the papillary dermis of a majority of CSU patients. This finding suggests that IgG or IgM autoantibodies in the skin are able to cause complement activation and supports the current hypothesis that IgG autoantibodies are involved in the pathogenesis of CSU.[79, 95] Complement-fixing autoantibodies and complement deposition in the skin are also frequently found in systemic lupus erythematosus (SLE), which may point to common pathomechanisms in CSU and SLE.[96] In SLE, C4d was found not only in blood vessel walls (80% of patients) but also along the dermo-epidermal junction (100% of patients). In the subacute cutaneous lupus erythematosus, deposits of C4d were detected within epidermal keratinocytes, and in pemphigus cases, intercellular C4d was found which roughly corresponded to the location of autoantibodies.[97] The location of C4d deposition – in the superficial dermis – is where infiltration was seen most in patients with urticaria. It is not surprising that C4d deposits were, in lower amount,

also present in non-lesional skin, as the presence of C4d deposition in non-lesional skin may be indicative of previous whealing – the non-lesional skin might in fact be post-lesional, since urticarial lesions tend to come and go – or it may point towards a widespread rather than local activation of the complement system. At present, it is unknown whether C4d deposition in urticaria is limited to the skin.

Furthermore, the correlation trend between C4d deposition and neutrophils scores and perivascular infiltration scores in the superficial dermis suggests granulocyte infiltration might be accompanied by local complement activation.

The role of complement in CSU is further supported by the fact that baseline C5a levels in peripheral blood were elevated in CSU patients compared to healthy individuals. Since it is known that complement activation and in particular C5a can be important for basophil activation in urticaria,[39, 98] these results support the role of complement in pathogenesis of CSU. We observed no correlation between the presence of C4d and C5a levels. This may be explained by the fact that C4d binds covalently and remains stable in structures surrounding endothelium, thus escaping early removal from the target organ whereas C5a is cleared rapidly. In patients recovering from acute humoral rejection after kidney transplantation, C4d deposition was cleared after 21 to 41 days,[99] in part due to plasmapheresis, which is continued until circulating IgG anti-donor HLA-antibody levels are sufficiently reduced. In our study we found a much faster decrease of C4d deposition in the skin. This situation is not comparable to acute humoral rejection as remaining IgG HLA-antibodies present in the kidney tissue will influence the rate of C4d clearance, whereas omalizumab reduces IgE and not IgG levels.[100] Since complement is mainly activated by IgG[101] , omalizumab treatment might not have an influence on the complement system as this drug exclusively binds IgE which cannot fix complement. This suggest that, in at least a proportion of patients with CSU, the relevance of complement in the pathogenesis is minimal.

Upon omalizumab administration complement activation was found within an hour and of C5a within two hours, after which all levels normalised within 6 hours. Immediate response of the complement system has been shown before in rituximab, omalizumab and OKT3 treatment where complement consumption could be observed already within 5 minutes after completion of rituximab infusion[94] and an increase in C3bc or C4bc was observed 30 minutes after onset of infusion.[102] Hence, this immediate complement activation is not specific to omalizumab. Complement activation at baseline may be caused by autoantibodies known to be commonly present in CSU patients although it must be noted that recent studies show contemporary IgE and IgG responses to the same autoantigens.[79, 95, 103-105] Little is known about these autoantibodies. The incidence of thyroid autoantibodies in patients with chronic urticaria is reported to range from 6.5% to 57% However, whether these antibodies predispose to autoimmune thyroiditis and hypothyroidism is not clear. Additionally, specific antinuclear antibodies have been

studied, but a low frequency of positivity was reported (2.5% of women and 0.9% of men), and again, the relation to clinical symptoms remains unknown.[106] Furthermore, as C5a normalization was not persistent throughout the study, we conclude that the early clinical responses observed after administration are not due to restoration of complement-mediated pathophysiology in CSU. The question remains how this temporary complement activation upon anti-IgE therapy can be explained. Baseline demographic characteristics were fairly similar to previous studies, and therefore we expect that our results are generalizable to the general CSU population in need of third-line treatment. Previously, Kolkhir et al reviewed the hypothesis of Type I and Type IIb autoimmunity in the pathogenesis of CSU.[107] The authors discuss that an IgG-anti-FcεRI/IgE-mediated activation of mast cells and basophils might be depended of complement C5a through its C5aR receptor. Furthermore, in SLE systemic formation of complexes of C1q and C3 with IgG is seen as deposition of immune complexes along the dermal-epidermal border, a phenomenon which might be reflected by C4d deposition in CSU patients.[96] The relatively small patient numbers and the absence of a multiple testing correction for the different complement components is a limitation of this study. Therefore, additional research in larger study populations are needed. In conclusion, both C4d deposition in lesional skin and elevated C5a levels in peripheral blood indicate the involvement of complement activation in the pathogenesis of CSU. No correlation was found between (response to) omalizumab and activation of complement, indicative of independent processes in the immunopathogenesis of CSU.

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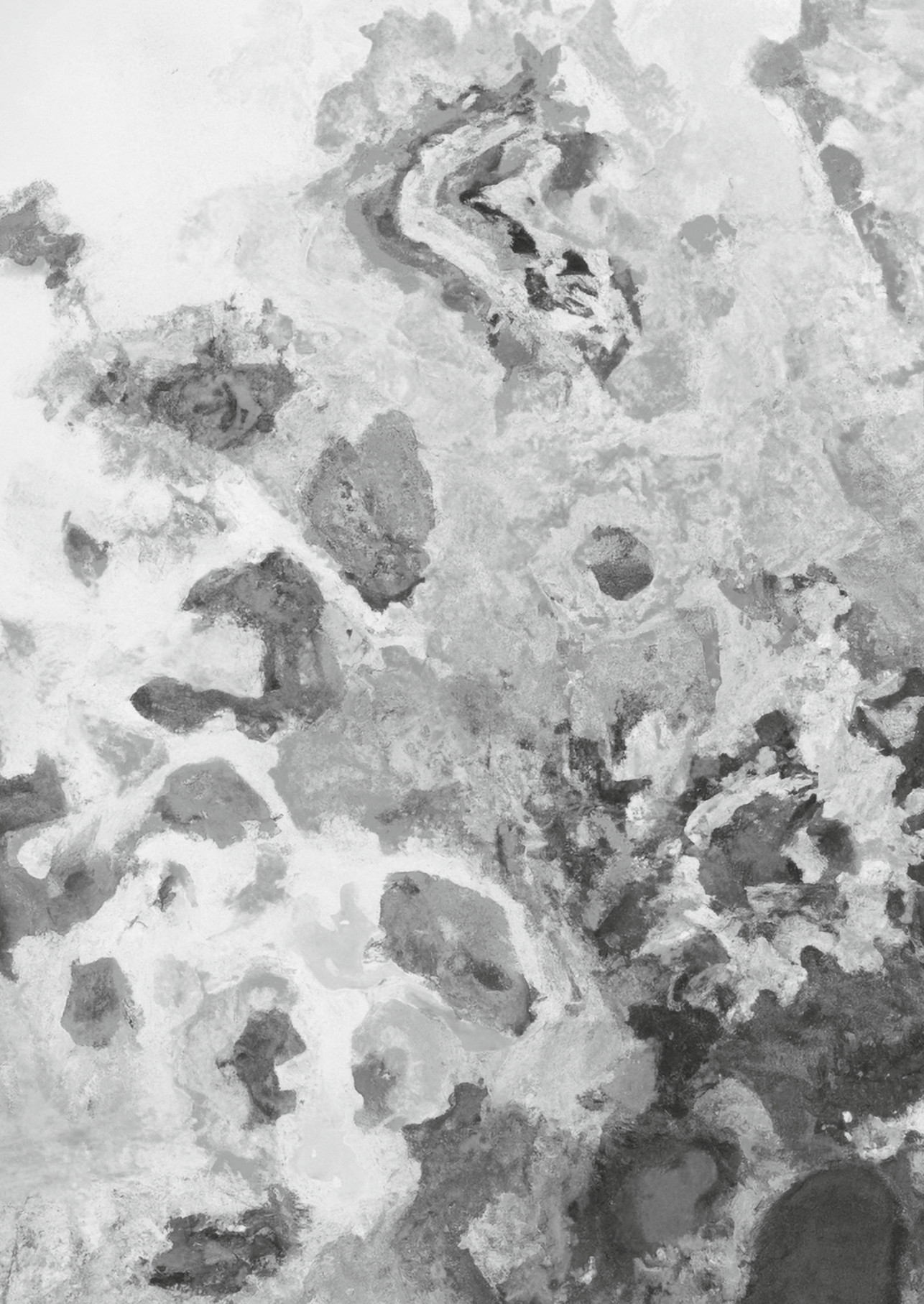
SUPPLEMENTAL TABLES AND FIGURES

Protein	Correlation with UAS7 ρ (p-value)
C1q	0.059 (0.765)
C3	-0.147 (0.454)
C3bc/C3	0.345 (0.072)
C4	-0.126 (0.524)
C4bc/C4	0.153 (0.438)
C5a	-0.038 (0.848)
MAC	0.159 (0.428)

Table E1: correlations between difference in disease activity after 1 week and difference in peripheral blood complement levels 1 hour after the first administration

For each protein Spearman's correlations were calculated between the difference in complement level from baseline after 1 hour (C5a: after 2 hours) and the difference in UAS7 score from baseline after 1 week.

Total number of measurements: 390 for C1q, C3, and C4, and 276 for C3bc, C4bc, C5a, and MAC.



CHAPTER 8

Response of FcεRI-bearing leukocytes to omalizumab in chronic spontaneous urticaria

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ABSTRACT

Background: The pathogenesis of chronic spontaneous urticaria (CSU) and the mechanism of action of omalizumab in CSU remains unclear.

Objective: In this study, we assessed the responsiveness and FcεRI expression of various subsets of leukocytes in patients with CSU treated with omalizumab.

Methods: In this prospective cohort study, 30 patients were treated with 6 administrations of 300 mg omalizumab every four weeks, followed by a follow-up period of 12 weeks. FcεRI expression as well as the percentage of basophils, monocytes, and dendritic cell subsets were analysed before and during treatment, and after follow-up. In addition, anti-IgE- and C5a-induced basophil degranulation was measured. The results were correlated to disease activity and response to omalizumab

Results: In addition to a rapid and significant reduction of FcεRI on basophils, we demonstrated a reduction of FcεRI on plasmacytoid dendritic cells during omalizumab treatment, which persisted until three months after discontinuation. FcεRI expression on basophils and its reduction did not correlate to the treatment response.Omalizumab led to an increased percentage of basophils in blood but not of the other FcεRI- bearing leukocytes. Basophil responsiveness was differentially affected; anti-IgE-, but not C5a-induced basophil degranulation increased during the treatment. Apart from clinical non-responders showing a stronger increase of anti-IgE-induced basophil degranulation over a period time, no differences were found in omalizumab-responders versus -non-responders.

Conclusions/Clinical Relevance: FcεRI expression on basophils decreased rapidly, while anti-IgE- induced degranulation significantly increased due to omalizumab treatment in patients with CSU, persisting at least for three months after stopping the treatment. None of the markers were able to predict the effectiveness of treatment. Whether basophils play a role in omalizumab responsiveness in CSU remains unclear.

INTRODUCTION

Chronic spontaneous urticaria (CSU) manifests as a skin disease with a sudden onset of wheals, which last longer than 6 weeks. The disease duration ranges from 1 to 5 years or even longer in more severe cases.[4] Omalizumab is effective as a third-line treatment in a majority of CSU patients with insufficient response to a fourfold dose of antihistamines.[70, 108] It is administered subcutaneously and reaches peak serum concentrations after an average of 7-8 days. Clearance of the monoclonal antibody is slow, with a terminal half-life of 19 - 22 days[109], which allows for relatively long treatment intervals of four weeks. A rapid clinical response can be seen in a proportion of the patients after the first omalizumab dose administration; however, other patients require multiple doses to reach a well-controlled disease status.[110]

Omalizumab is a humanized monoclonal antibody, which binds to the Cε3 domain of free IgE, thereby preventing it from binding to Fc epsilon RI (FcεRI).[111] Depletion of free IgE by omalizumab leads to a down-regulation of the FcεRI on mast cells in a majority of patients.[81, 112] Mast cells are considered to be the most important effector cells in CSU. In addition, a role for basophils has been suggested in certain urticaria phenotypes.[113] Basophil numbers are inversely related to urticaria severity.[114, 115] An increased presence of basophils in the skin and decreased numbers in peripheral blood suggest that basophils are recruited to the affected skin sites.[32, 116, 117]

Recent studies investigating the response of skin mast cells to omalizumab in allergic patients showed down-regulation of FcεRI expression after 1-2 months.[33, 118, 119] Therefore, other cell types, such as basophils and dendritic cells, might account for the rapid clinical effect of omalizumab.[78, 120]

Decreased degranulation of basophils after stimulation via FcεRI was demonstrated in patients with urticaria compared to that in healthy controls.[114] It is not known if responses to other stimuli, such as C5a (which activates basophils via a G-protein-coupled pathway) is affected.[121] Besides basophils and mast cells, other myeloid cells can also express FcεRI on their surfaces.[122] The presence of FcεRI has been demonstrated on monocytes and different types of dendritic cells, more profoundly in patients with allergies than in healthy individuals. In allergic rhinitis patients, expression of FcεRI on the different myeloid cells depended on serum IgE concentration, and treatment with omalizumab reduced the expression of FcεRI on basophils, mast cells, and DCs.[34, 123, 124] Recently, Deza et al. suggested that the baseline expression of basophil FcεRI was a potential immunological predictor of responsiveness to omalizumab in urticaria. Furthermore, they found that patients who responded to omalizumab treatment had a rapid reduction in the levels of basophil FcεRI during treatment. Given the potential role of FcεRI-bearing leukocytes, in particular basophils, in the pathogenesis and/or

treatment response to omalizumab, we evaluated the role of FcεRI-bearing cells in CSU patients treated with omalizumab.

METHODS

Design and population

This monocentric exploratory prospective cohort study was performed in the University

Medical Center Utrecht, the Netherlands, from 2015 until 2017. We included 30 patients according to the following criteria: age ≥ 18 years, active diagnosis of CSU (weekly urticaria activity score [UAS7]) ≥ 16, in-clinic UAS ≥ 4 on the day of the first omalizumab administration, and insufficient response to a four-times daily administration of antihistamines. Exclusion criteria were: clearly defined underlying aetiology for chronic urticaria (e.g. chronic inducible urticaria [CINDU]), a history of malignancy, known hypersensitivity to omalizumab, and pregnancy. Routine administration of immunosuppressants, including prednisolone and Cyclosporine A (CsA) was discontinued with washout periods of 3 months prior to treatment with omalizumab. If prednisolone was used as a rescue medication, a washout period of 2 weeks was maintained.

After a screening period of up to 2 weeks, eligible patients received six doses of 300 mg omalizumab every four weeks. After the last omalizumab administration, the patients were observed during a follow-up period of three months. The patients were kept on a treatment with fourfold dose of H1 antihistamines throughout the study period. As a rescue medication, patients were allowed to use prednisolone, up to 30 mg daily. All other CSU-related medications were discontinued.

Disease activity was measured throughout the study using the UAS7.[93] Treatment response is defined as a UAS ≤ 6 at week 24 of treatment. Improvement by a minimal important difference (MID) is defined as a reduction of 10 UAS7 points.[56] All the patients provided written informed consent, and the study was approved by the local ethics committee (protocol number 15-167).

*Blood collection*Blood samples were collected at the following time-points: at baseline (T0) and at different time points after the first injection: 6 hours (T0.25), one day (T1), one week (T7), two weeks (T14), one month (second dose, T28), one month and two h (T28.08), two months (T56), three months (T84), four months (T112), five months (T140) and eight months (follow-up, T224). EDTA blood and gel separated serum were placed on ice immediately after venipuncture. Blood was also collected from nine self-reported healthy controls for baseline analysis. All serum samples were allowed to coagulate for 60 min. Serum and plasma were obtained by centrifugation and stored at -80 ºC. Total IgE was determined using the ImmunoCap assay according the manufacturer's instructions (Thermo Fisher Scientific, Uppsala, Sweden).

Leukocyte subset determination

Leukocytes subsets were identified using an antibody panel containing CD45-PO (Life Technologies) for lymphocytes; CD123-PerCPCy5.5 (BD Pharmingen), CD203c APC (Sony), HLA-DR-PB (Sony), and CD41-PE-Cy7 for basophils; and CD45-PO (Life Technologies) and CD14-APC-H7 (BD Pharmingen) for monocytes. To distinguish between the three different subsets of dendritic cells (DCs), an antibody panel containing HLA-DR-PE-CY7 (Biolegend), CD11c-PB (Biolegend), and CD123 PerCP Cy5.5 (BD Pharmingen) was used for plasmacytoid dendritic cells (pDCs). For two subsets of myeloid dendritic cells (mDCs), CD14-V500 (BD), HLA-DR-PE-CY7 (Biolegend), CD1c-APC-Cy7 (Biolegend), and CD141-APC (Milteny) were used. Leukocyte subset quantities were depicted as the percentage of cells within the total leukocyte measures/numbers.

Quantification of FcεRI expression

Whole blood samples were divided into aliquots of 75 µl each to carry out staining of basophils, monocytes, dendritic cells (DC), or an isotype control. All the cells were stained for either FcεRI (CRA-1, eBiosience) or an IgG2b isotype control (Sony) for 30 min at 4°C in the dark. Following washing, cells and QIFIKIT beads (Dako, Glostrup, Denmark) were simultaneously stained with a saturated solution of goat anti-mouse IgG FITC to determine the absolute FcεRI expression, quantified as antibody-binding-capacity (ABC). The QUIFIKIT contains five bead populations with a distinct and known amount of monoclonal mouse antibody bound per microsphere bead. By constructing a calibration curve based on the fluorescence intensity of different populations plotted against their known antibody density, FcεRI expression on different cell types can be interpolated based on their mean fluorescence intensity (MFI). The specific antibody-binding capacity (SABC) is then calculated by subtracting the calculated ABC for corresponding isotype controls from the anti-FceRI ABC.

Basophil activation test

Heparin-anti-coagulated blood samples were stimulated for 30 min at 37°C with increasing concentrations of anti-IgE (0.03 µg/ml, 0.1 µg/ml, 0.3 µg/ml, and 1 µg/ml) (Vector laboratories) or C5a (83 ng/ml and 200 ng/ml) (R&D Systems) in RPMI-1640 medium (Gibco, Life Technologies) containing 1 ng/ml IL3 (R&D Systems). Leukocytes were stained with an antibody cocktail of CD45-PO (Life Technologies), CD123-FITC (Biolegend), HLA-DR-PB (Sony), CD63-PE (Monosan), CD41 PE-CY7 (Beckman Coulter), or CD203c-APC (Sony). Basophils were defined as $CD45^{+}$ $CD203c^{+}$ $CD123^{+}$ and $HLA\text{-}DR^{-}$ $CD41^{-}$. Basophil degranulation was quantified by determining the percentage of CD63-binding basophils. The threshold for basophil degranulation was set between degranulated and resting basophils.

Statistical analysis

Differences in cell counts, basophil activation test (BAT) results, and FcεRI density in time were analysed using Wilcoxon matched pairs signed rank tests. Analyses between

different responder groups were performed using Mann-Whitney U tests. Correlation analysis was performed using Spearman rank correlation or Pearson correlation if appropriate. Regarding the UAS7 score, the difference between each time-point and baseline was tested using Wilcoxon matched pairs signed rank tests. Statistical analysis was performed using IBM SPSS Statistics version 21 or GraphPad Prism version 7.02. Graphs were plotted using Microsoft Visio 2010 or GraphPad Prism version 7.02.

RESULTS

Clinical efficacy of omalizumab

Thirty patients [median age 42 years (range 21-700; 73 % female)] with a median UAS7 score at baseline of 31.5 points were enrolled in the study. Patient characteristics are similar to the previous published article from our clinic as well as current studies in literature.[9]

Figure 1 shows the weekly median values of UAS7; the patients were differentiated into omalizumab-responders and -non-responders. Fifteen patients (50 %) showed a UAS7 score of six or lower (median 0) at four weeks after the last omalizumab administration (24 weeks) and were defined as responders. Fourteen patients showed a UAS7 score of seven or higher (median 16) at week 24 and were defined as non-responders. The UAS7 score of one patient was missing at week 24 and was marked as non-responder based on the last known UAS7 score.

Improvement by a minimal important difference (MID) of 10 UAS7 points at week 24 was observed in 23 patients (76.6%), which included nine complete responders (UAS7 =0).

Due to worsening of the disease, 11 patients, of which 6 (55%) were presented as responders, restarted omalizumab treatment during follow-up. Subjects who restarted omalizumab during the follow-up period were excluded from the follow-up data analysis. In absolute numbers, the number of patients who were excluded were: 1 in week 25, 2 in week 26, 3 in week 28, 4 in week 29, 9 in week 30, and 11 in week 32.

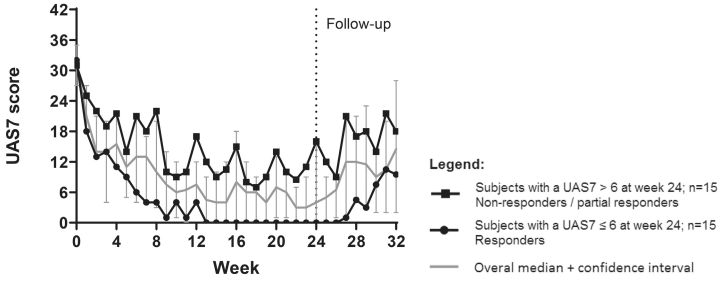

Figure 1 : Median values of UAS7 for responders and non-responders improve during omalizumab treatment. Median values of UAS7 at baseline, during omalizumab treatment, and during follow-up are presented for responders and non-responders. At the start of week 20, the final dose of omalizumab was administered, which initiated the follow-up period after week 24 (dotted line). Subjects who restarted omalizumab during the follow-up period were excluded from data analysis.

FcεRI expression on basophils, pDCs and mDC CD1cs decreases during treatment

In peripheral blood, we determined FcεRI expression on basophils, monocytes, pDCs, and two subsets of mDCs (mDC CD141 and mDC CD1c) at specific time points before, during, and after treatment. A large and significant difference in FcεRI expression on basophils was found at T7 ($p<0.0001$) and all other time points, including after 3-month follow-up (T224) compared to that at baseline (T0) (Figure 2). Reduction of FcεRI expression did not differ significantly between responder and non-responder groups. The decline in FcεRI expression showed a weak correlation

with the decline in UAS7 score, one week after baseline ($r = 0.675$, $p = 0.008$). A similar decline in FcεRI expression after omalizumab treatment was observed for pDC and mDC CD1c (data not shown).

Furthermore, FcεRI expression on basophils at baseline did not differ between omalizumab responders and non-responders ($p=0.202$), healthy controls and responders ($p=0.215$), and healthy controls and non-responders ($p=0.682$) (Figure 2A and B). Moreover, we did not find a statistical difference ($p=0.408$) when comparing extremes response groups: complete responders (n=9, all UAS7=0) to extremely poor responders (n=7, UAS>16; median UAS7 = 25).

Total IgE (available for 28 of the 30 patients) did not differ significantly either between responders (n=15, median: 170.0 kU/L) and non-responders (n=13, median 81.9 kU/L; $p=0.387$) or between patients with self-reported atopy (n=15, median: 122.0) and without atopy (n=13, median: 107.0, $p=0.467$). In addition, no correlation was found between total IgE levels and baseline basophil FcεRI expression ($r=0.081$, $p=0.682$). However, when removing one extreme outlier with a total IgE > 5000 KU/L, a moderate correlation between baseline total IgE and baseline basophil FcεRI expression ($r=0.4$, $p=0.037$) was seen, which was comparable to that mentioned in a recent study.[125]

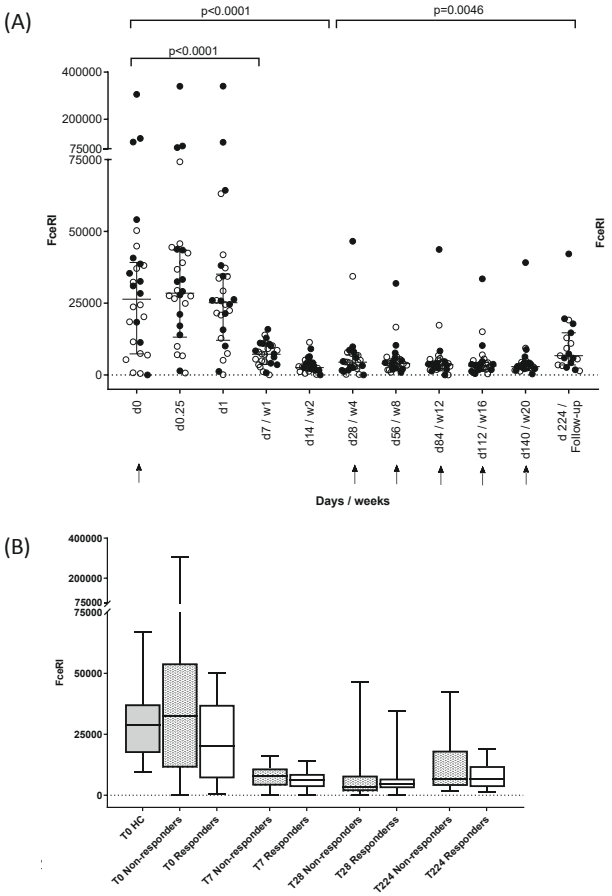

Figure 2: Median FcεRI expression on basophils decreases during omalizumab treatment.
(A) FcεRI expression on basophils at various time points.
Responder : ○
Non-responder : ●
Arrows indicate omalizumab administration. Count in molecule median per basophils.
(B) FcεRI expression on basophils at selected time points in healthy controls (HC) (grey box-plot), non-responders (dotted box-plot) and responders (white box-plot).

Only basophils percentages increase, other FcεRI-bearing leukocytes remain stable

We measured percentages of basophils, monocytes, pDCs, mDCs (CD141) and mDCs (CD1c) at baseline (T0), several time points during omalizumab treatment, and at 3-months follow-up (T224). Median percentage of basophils measured in blood showed an increase during omalizumab treatment (figure 3) within one day (median: 0.18) compared to baseline (median: 0.13), reaching a maximum at four weeks. At follow-up, the median percentage of basophils (median: 0.24) was still higher compared to that of baseline. Healthy controls showed a higher percentage of basophils compared to CSU patients at baseline ($p<0.001$). There was no difference in median percentage of

basophils between non-responders and responders at any given time point. However, we did notice a significantly faster increase in the percentage of basophils one week after the first omalizumab administration in the responder group compared to that of the non-responder group [p=0.011, (Figure 3B)].

There was no significant change in median percentages of the other analysed leukocytes, such as eosinophils, monocytes, pDCs, mDC CDC141^{+}, and mDC CD1c^{+} after omalizumab treatment (data not shown).

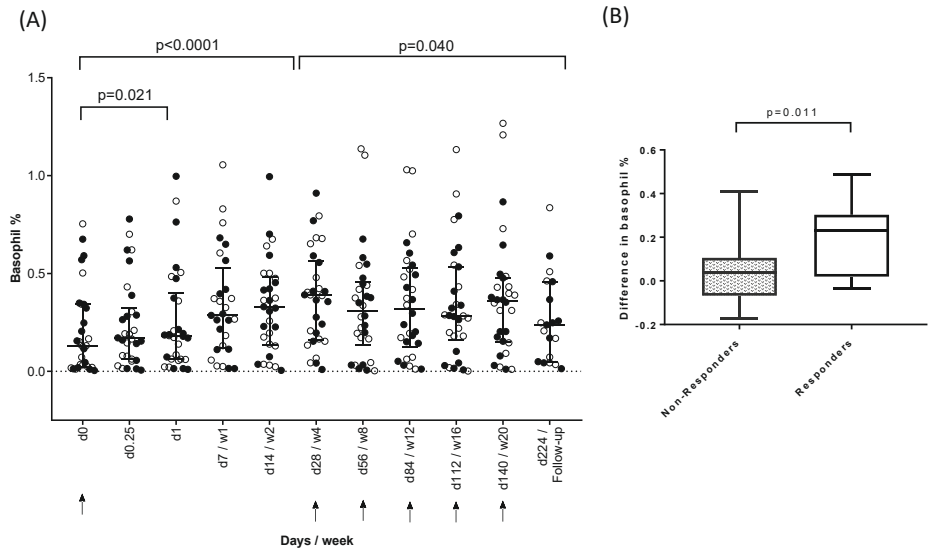

Figure 3: Percentage of basophils measured in a patient's sample increase during omalizumab treatment. (A) Portion of basophils measured at various time points. (Responder : ○, Non-responder : ●). Arrows indicate omalizumab administration. (B) Basophil percentage after one week of omalizumab treatment compared to that at baseline in omalizumab non-responders (dotted box-plot) and responders (white box-plot) at week 24.

Anti-IgE- but not C5a-induced basophil degranulation increases during omalizumab treatment

A significant increase in anti-IgE-induced (1 µg/ml) basophil activation was observed after 24 h (p=0.042), which was maintained for all the subsequent time points (Figure 4). A similar pattern was seen after stimulation with suboptimal concentrations of anti-IgE (0.3, 0.1, and 0.03 µg/ml). A significant difference was not observed in baseline anti-IgE-induced basophil degranulation between responders and non-responders (p=0.148). However, non-responders showed a significantly stronger increase of anti-IgE-induced basophil activation at T28 compared to T0 (p=0.003) and at T224 compared to T28 (p=0.049), while responders did not show a significant difference (p=0.104 and p=0.742 respectively) (supplemental figure 1).

Contrary to the anti-IgE–induced FcεRI-mediated basophil degranulation, C5a-induced degranulation showed a significant reduction (figure 4) after one month of treatment (p=0.025) and at all subsequent time points during treatment. This reduction in C5a-induced basophil degranulation returned to baseline levels during the follow-up period. Responders and non-responders showed a similar pattern of C5a-induced basophil degranulation. When basophils were stimulated with a suboptimal concentration of 83 ng/ml, a similar effect was observed as that with stimulation with an optimal concentration of C5a of 200 ng/ml.

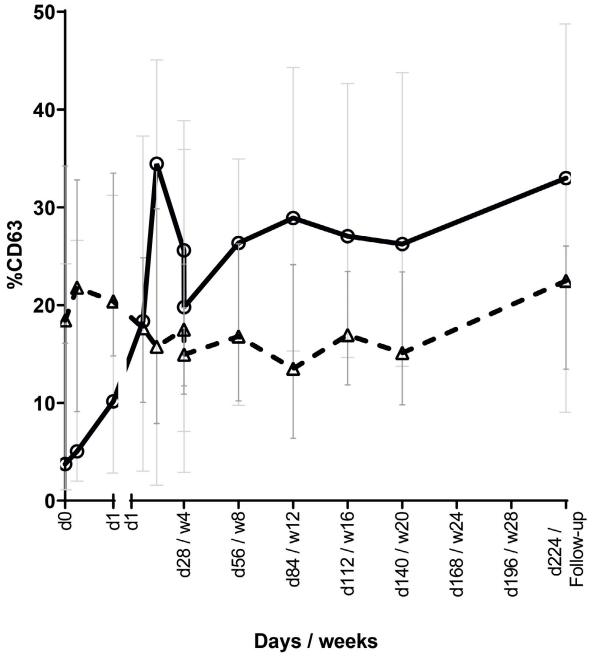

Figure 4: Anti-IgE-induced basophil activation (1 µg/ml) increases and C5a-induced basophil activation (200 ng/ml) decreases during omalizumab treatment.
Basophils were stimulated with 1 µg/ml anti-IgE (-O-) or 200 ng/ml C5a (-Δ-) for 30 min and activation was determined as % cells with increased CD63 expression by flow cytometry.

DISCUSSION

In this study, the effect of omalizumab treatment in CSU on different FcεRI-bearing leukocytes was investigated.

A decline of FcεRI on basophils was observed within one week after initiation and during omalizumab treatment, as shown in earlier studies.[81, 112] In this study, we present several important and novel findings. The decreased FcεRI expression on basophils persisted up to 12 weeks after the last dose and was also detected on the mDC CD1c subset and pDCs. However, this decrease was not found on monocytes or on the mDC CDD141

subset. We also observed that the basophil responsiveness was differentially affected by omalizumab treatment, since anti-IgE-, but not C5a-induced basophil degranulation increased during the treatment.

Another important finding was that we could not relate either the baseline expression or the decline of FcεRI expression on basophils to the clinical effect of omalizumab. A recent study[28] suggested that baseline FcεRI expression could predict omalizumab treatment response. In a study of Deza et al, non-responders showed significantly lower baseline levels of FcεRI expression compared to healthy controls and responders. Furthermore, a decrease in FcεRI expression was mainly observed in responders. In our study, we found a large overlap in the FcεRI levels between healthy controls, responders, and non-responders, and no statistical difference was found among the three groups. Moreover, we did not find a statistical difference (p=0.408), when complete responders (n=9, median UAS7=0) were compared to extreme poor responders (n=7, UAS>16, median: 25). Moreover, the change in FcεRI levels did not correlate with the change in urticaria activity (UAS7 scores) per patient. A possible explanation for the discrepancy between the two study results might be a difference in patient population. Although demographics between the two studies were fairly similar, patient-reported outcomes differed noticeably, since 81% of the patients in the study by Deza et al. achieved a UAS7 score of ≤ 6 or a ≥ 90% reduction in UAS7 versus only 50% in our study.

The decreased FcεRI expression levels persisted in patients for at least 3 months even after discontinuing the omalizumab treatment. In a similar study, Jörg et al found that FcεRI expression on basophils was decreased during omalizumab treatment and up to 2 months after the last dose.[126] These results suggests lasting effects of omalizumab, which might explain why a proportion of the patients show a beneficial effect even after long intervals.[14]

The median percentage of peripheral blood basophils showed a rapid and significant increase over time (figure 3), which has been described previously during omalizumab and steroid treatment.[29, 112, 115] None of the other leukocytes showed such a reaction to omalizumab treatment, which may support a prominent role of basophils. There was no difference between omalizumab responders and non-responders. However, a significantly stronger increase in the percentage of basophils was observed in responders compared to non-responders solely one week after the first administration of omalizumab.

These findings point towards a possible compartmental shift, in which basophils remain in the circulating blood rather than migrate to the affected skin, as suggested by Grattan et al.[115] In our study, 4 of the 30 patients (1 responder, 3 non-responders) reported prednisolone use at some time-points during the study period, which might have influenced the blood basophil numbers.

An interesting new finding was that the degranulation of basophils was significantly increased after the crosslinking of FcεRI to anti-IgE, despite the strongly diminished expression of FcεRI on basophils by omalizumab. The low pre-treatment level of degranulation of the basophils might point towards a refractory state of basophils due to activation in CSU. The increase in degranulation was seen in both optimal and suboptimal concentrations of anti-IgE.

Simultaneously, we found that C5a-induced degranulation of the basophils was slightly decreased due to omalizumab treatment. This indicates that the observed increase in anti-IgE- induced degranulation was not an overall increase of intrinsic basophil sensitivity but was specific for the FcεRI selective activation routes. This can most probably be explained by the different stimulus-secretion pathways that are used by FcεRI versus G-protein-coupled C5a receptors.[127, 128] Our findings are in line with a recently described study by MacGlashan and Saini on cat allergic individuals treated with omalizumab.[129] This study described that an increased intrinsic basophil sensitivity was the underlying cause of increased IgE-mediated degranulation of basophils, which was later suggested to be the result of an omalizumab-induced increased expression of Syk in basophils.[130] Why this selective responsiveness of basophils changes after omalizumab treatment is unclear, however it does emphasize the important role of basophils in the mechanism of CSU. We speculate that it might be a reflection of the different maturation state of basophils due to decreased tissue inflammation, which in turn reduces the number of basophils in the skin and potentially leads to a lesser amount of basophil differentiation in the bone marrow.

Neither anti-IgE- nor C5a-induced basophil activation was related to treatment response. However, increase of anti-IgE mediated basophil activation was most apparent in samples from patients not responding to omalizumab (supplemental figure 1). None of the other cellular responses showed a significant difference between responders and non-responders, therefore we were not able to elucidate their role in the omalizumab treatment. These findings imply that the omalizumab-induced basophil changes might be responsible for the underlying clinical effects, but a yet unknown additional cellular effect could also play a role towards the favourable clinical response.

Notably, both the decrease of FcεRI expression on the cell surface of basophils, and the increase of anti-IgE mediated basophil stimulation and decrease of C5a-mediated basophil stimulation continued for at least 3 months after discontinuation of omalizumab. Given the relative short lifespan of basophils, this suggests either a prolonged effect of omalizumab or involvement of a more complex (possibly intracellular) mechanism of action of omalizumab.

Omalizumab induced a rapid and sustained decline of FcεRI expression on the surface of basophils, pDCs, and mDC CD1c. Despite the diminished expression of FcεRI on

basophils by omalizumab, basophil degranulation was significantly increased after the crosslinking of FcεRI to anti-IgE. However, none of the findings could predict the response of omalizumab treatment, and more research is therefore required. Our findings suggest that response to omalizumab in CSU patients may be partly explained by pathways involving a high-affinity IgE receptor of the basophils.

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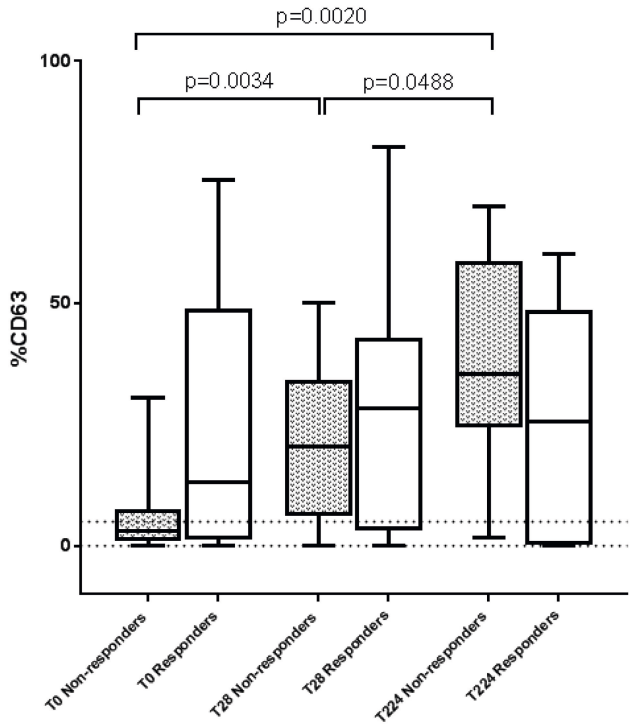
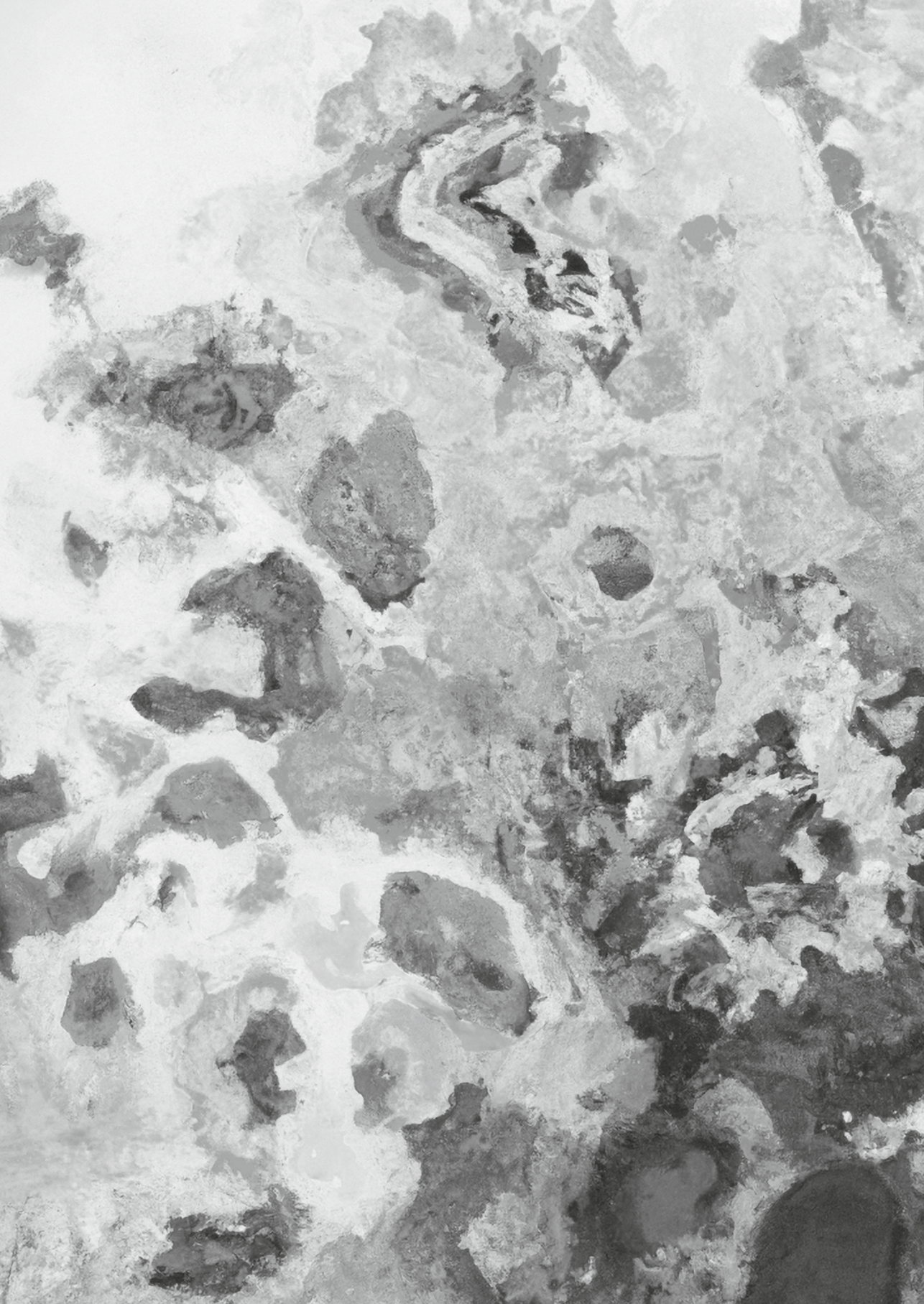
SUPPLEMENTAL TABLES AND FIGURES

Characteristic	Frequency or range
Median age in years (range)	42.0 (21 - 70)
Female, n (%)	22 (73)
Weight in median kg (range)	83.7 (61.4 – 114.2)
Body mass index median kg/m2 (range)	27.2 (21.2 – 44.6)
Presence of angioedema, n (%)	24 (80)
Presence of CINDU in addition to CSU, n (%)	12 (40)
Delayed pressure urticaria	8 (27)
Urticaria factitia	6 (20)
Family history of wheals or angioedema, n (%)	7 (24)
Median disease duration in years (range) , n (%)	2.7 (0.6 - 29)
Atopy by history, n (%)	
Any atopy	17 (57)
Atopic dermatitis	10 (33)
Asthma	5 (17)
Allergic rhinitis	9 (30)
Other allergy	8 (27)
Medication use on day of OMA1, n (%)	
Second generation antihistamines (sgAH)	30 (100)
First generation antihistamines (fgAH)	3 (10)[†]
H2-antagonist	0 (0)
LTRA	6 (20)
H1-antihistamine dose on day of OMA1, n (%)	
Threefold	2 (7)
Fourfold	28 (93)
Previous switch of type of sgAH, n (%)	17 (57)
Previous use of systemic steroids, n (%)	14 (47)
Previous use of immunosuppressants, n (%)	6 (20)
Number of subjects with UAS7 ≤ 6 after one month	6/30 (20)
Number of subjects with UAS7 ≤ 6 after six months	15/30 (50)

Supplemental table 1: Patient and disease characteristics
Note that 2 patients with CINDU experienced more than one subtype; and therefore, numbers do not tally within the table. [†]fgAH were exclusively administered as a demand medication. OMA1: day of first omalizumab administration.



Supplement Figure 1: Anti-IgE-induced basophil activation (1 µg/ml) in non-responders increases more rapidly compared to that in responders.
In responders: T0 vs T28: p=0.0034, T28 vs T224: p=0.0488, T0 vs T224: p=0.0020.
In non-responders: T0 vs T28: p=0.1040, T28 vs T224: p=0.7422, T0 vs T224: p=0.6406.



CHAPTER 9

Recombinant human C1 esterase inhibitor as prophylactic treatment in idiopathic non-histaminergic angioedema

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ABSTRACT

Background Idiopathic non-histaminergic acquired angioedema (InH-AAE) does not respond to anti-histamines and is possibly mediated by bradykinin.

Objective To investigate the efficacy and safety of recombinant human C1-esterase inhibitor (rhC1-INH), for prophylaxis of InH-AAE, and to evaluate contact system parameters as biomarkers for attacks.

Methods A prospective, open-label study of patients with InH-AAE with > 2 AE attacks/month. rhC1-INH (50 IU/kg was administered intravenously; maximum 4200 IU) twice weekly for 2 months, preceded and followed by a one-month period of observation. The primary endpoint was a >50% reduction in attack frequency. C1INH-function and plasma levels of high molecular weight kininogen, factor XII, plasma prekallikrein, C4, cleaved kininogen, d-dimer were evaluated as potential biomarkers. Post-trial follow-up was evaluated in all patients.

Results Six patients (mean age 41 years; four female) were enrolled. One patient showed a reduction in attack frequency of 85% (3 versus 20) during rhC1-INH treatment, and showed clinical response to plasma-derived C1-INH but not to omalizumab post-trial. Restarting rhC1-INH treatment resulted in a similarly rapid response. The other 5 patients showed no improvement. No major adverse events were reported. None of the measured biomarkers were related to treatment response. Post-trial, omalizumab was administered to four patients, of which two reported response.

Conclusion rhC1-INH treatment was effective in 1 of 6 InH-AAE patients, suggesting a bradykinin-dependent mechanism of attacks in this patient. Response to omalizumab or tranexamic acid during follow-up in a number of the patients points to a heterogeneous pathogenesis of InH-AAE disease requiring a personalised treatment approach.

INTRODUCTION

Angioedema (AE) is characterized by episodes of acute submucosal or subcutaneous swellings,[131] and strongly influences quality of life.[132] The pathophysiological mechanisms of AE are not yet fully understood. Generally, two vasoactive peptides are thought to mediate acute AE swellings; namely, histamine and bradykinin. Histamine is released from activated mast cells and basophils, possibly through activation of the high-affinity IgE receptor, and is associated with allergic AE and AE in patients with chronic spontaneous urticaria.[80, 132] However, this pathway is not fully understood and more mediators other than histamine are possibly involved in mast-cell mediated angioedema[38]. Bradykinin is generated upon activation of the plasma contact system, which comprises of factor XII (FXII), plasma prekallikrein (PK) and high molecular weight kininogen (HK). Active FXII (FXIIa) activates PK into plasma kallikrein (PKal) that cleaves bradykinin from HK.[133] By binding to mainly the bradykinin receptor 2, bradykinin increases vascular permeability and mediates AE.[134] The bradykinin pathway mediates hereditary AE (HAE) and angiotensin converting enzyme (ACE) induced AE.[134]

The pathomechanisms of attacks in idiopathic AE are less clear. 64-84% of patients with idiopathic AE respond to antihistamine treatment suggesting histaminergic AE in most of these patients.[135-137] Patients that do not respond to antihistamine therapy are considered as idiopathic non-histaminergic AE (InH-AAE), however this definition does not take into account the effect of omalizumab on the histaminergic pathway.[41, 138] Elevated bradykinin levels were found in four patients with InH-AAE during acute attacks compared to normal levels during remission and in healthy controls.[48] Measurements of bradykinin as biomarker are challenging due to its short half-life and high sensitivity to pre-analytical procedures. Therefore several other contact system parameters have been proposed as biomarkers, including complexes between C1-INH and contact system proteases (FXIIa, PKal), HK antigen levels, and cleaved HK (cHK).[139] The fibrin breakdown product D-dimer is also elevated in HAE[140] and CSU[141] and appears to reflect a condition with increased vascular permeability.

In HAE due to C1INH deficiency, C1-INH is supplemented in order to inhibit coagulation factor XIIa and PKal, preventing excess generation of bradykinin.[142] Restoring functional C1-INH levels above the 40% threshold has been reported to protect against AE attacks.[143] Effectiveness of C1-INH therapy has also been reported in patients with hereditary angioedema with normal C1 inhibitor. Three case-studies reported a complete (n=2) or partial (n=1) effect of prophylactic plasma-derived C1 esterase inhibitor (pdC1-INH). Icatibant showed effect within two hours in three of these described patients.[45-47]

Recombinant human C1 esterase inhibitor ((rhC1-INH), Conestat alfa/Ruconest®), is an effective and safe treatment for acute attacks and prophylaxis of AE in HAE type 1 and 2.[144] Given the unresponsiveness to antihistamine treatment in patients with Inh-AAE,

indicating possible involvement of the bradykinin route, the objective of our study was to investigate the effectiveness and safety of rhC1-INH prophylaxis in InH-AAE in a prospective clinical trial. Furthermore, we evaluated if plasma levels of C1-INH, C1-INH function, C4, FXII, PK, HK, and cHK, markers of the bradykinin pathway can be used to predict a therapeutic response to rhC1-INH in InH-AAE.

METHODS

Study participants

This phase 2 explorative, prospective, single-center, open-label study was performed from March 2018 to February 2021 at the dermatology and allergology department of the University Medical Center, Utrecht in the Netherlands.

Patients aged 18 years and older suffering from AE attacks with at least two attacks per month during the last six months despite prophylactic treatment with four times the standard daily dose of antihistamines, and no known cause for AE were eligible for inclusion. Additionally, C4 levels and C1-esterase inhibitor (C1-INH) function of functional levels were required (C4>0.1 g/L and C1-INH function > 0.63 U/ml). Patients were excluded in cases of accompanying wheals; pregnancy or breastfeeding; a history of rabbit allergy; ACE-inhibitor use in the past six months; recent or current use of methotrexate, azathioprine, mycophenolic acid, omalizumab or cyclosporine. Patients with clinically relevant conditions that had the potential to compromise the safety of the patient such as renal or hepatic insufficiency or malignancies or when another diagnosis was deemed more likely (e.g. allergic AE, drug-hypersensitivity, mastocytosis or HAE) were also excluded. All patients continued to use 4dd antihistamines in order to reduce the risk of bias due to alterations in treatment other than the initiation of rhC1-INH. Patients were allowed to use rescue medication during acute AE attacks including antihistamines, oral steroids and intramuscular adrenaline.

All patients provided written informed consent, and the study was approved by the local ethics committee (protocol number 17-139).

Study design

All enrolled patients completed a four-week observation period, followed by an eight-week treatment period with rhC1-INH (Recombinant human C1 esterase inhibitor (rhC1-INH), Conestat alfa/Ruconest®; Pharming Technologies; Leiden , The Netherlands), followed by another four-week observation period (Supplemental figure 1). Throughout the entire trial, patients continued using 4 doses daily antihistamines. The total dose of rhC1-INH was calculated based on body weight (50 IU/kg; max 4200 IU) and was twice-weekly administered intravenously over a time course of five minutes.

A detailed medical history and physical examination were recorded at first visit and adverse events and concomitant drug use were registered at each following visit.

The attack frequency and severity were recorded in the AE activity score (AAS) form.[145] Weekly AAS results (AAS7) were combined into AAS per four weeks (AAS28). An attack was separated from a subsequent attack when there had been an AE free period between reported swellings on subsequent days as scored on the AAS form. The Angioedema Quality of Life score (AE-QoL) was used to assess the effect of AE on quality of life,[146] with 0 to 23 indicating "no effect", 24 to 38 indicating a "small effect" and ≥ 39 indicating a "moderate to large effect".[147] The primary endpoint of the study was a reduction in attack frequency of 50% in the treatment period compared to the cumulative observation period. Secondary outcomes were AAS28 and AE Quality of Life (AE-QoL) scores during the treatment period compared to observation period. Outcomes were assessed per case. Post-trial follow-up data was collected by analysing patient records. InH-AAE patients were treated according to the AE and urticaria local treatment protocol,[54] which is in line with international guidelines, but shared decision between the patient and physician allowed for protocol deviation.

Collection of blood samples and laboratory assessments

Blood was obtained at visit 2,4 and 17 (prior to C1-INH dose administration) and at 18 (follow-up visit). C1-INH function was measured with a chromogenic assay (Sanquin, the Netherlands). C4 levels and total IgE were determined at visit 1. In women <50 years of age, pregnancy was excluded via a urine dipstick βHCG test. C-reactive protein (CRP), d-dimer and leukocyte count were determined with routine assays. C1-INH, FXII, PK and HK levels were visualised using immunoblot. For this, EDTA plasma was diluted 40 times in four times reducing sample buffer (15.5% glycerol, 96.8 mM Tris-HCL, 3.1% SDS, and 0.003% bromophenol blue, 25 mM DTT), boiled for 10 minutes, and 5 µL per sample was loaded and ran on a 4-12% Bis-Tris gel at 165V for 60 minutes and transferred onto Immobilon-FL membranes at 125V for 55 minutes. For detection, polyclonal goat anti human IgG antibodies (anti-human FXII Cl20055AP, anti-human PK Cl20090A, anti-human HK Cl20027AP, anti-human C1-INH CL200323AP, Cedarlane, Burlington, Canada) and Alexa Fluor 680 donkey anti-sheep IgG (lot#1878516, Dako, Glostrup, Denmark) were used.

Levels of cHK in EDTA plasma, an indirect marker for bradykinin release, were determined with ELISA as described above.[148] The upper normal limit was assessed using values of ~50 healthy individuals for cHK and ~20 healthy controls for C1-INH complexes.

Data analysis

Due to the small sample size, data was analysed using descriptive statistics.

Statistics and graphical demonstration of clinical data and laboratory outcomes were performed with GraphPad Prism 8.3.0 software.

Role of the funding source

The study was designed and performed by the study team of the UMC Utrecht at the Dermatology and Allergology clinic, and partly financed by Pharming Technologies, which was informed about the study protocol and was notified regarding inclusion of patients and progress in order to organize drug delivery. The company donated the required rhC1-INH for the treatment period and also donated rhC1-INH for patient 1 for six months treatment after the last study visit. Omalizumab treatment was given under standard insurance coverage.

RESULTS

Clinical response to rhC1-INH in one out of six patients

Six patients were included in the study, four females and two males. C1-INH function and C4 levels were normal in all patients. At the start of the study, the mean age was 41 years and the median disease duration was 8 years. Self-reported AE attack frequency in the six months prior to the study varied from 2.6 attacks to 15.8 attacks per month. Other baseline characteristics are presented in table 1. None of the patients had been treated with long term immunosuppressive treatment (methotrexate, azathioprine, mycophenolic acid, cyclosporine), Icatibant or omalizumab before the study.

	Weight (kg)	Duration of disease (months)	Atopy	Profylactic treatment1	Attack intervention1	Attack frequency (attacks/ month)	Baseline C1-INH function (U/ml)	Baseline C4 level (g/L)	Baseline total IgE (kU/L)	Dose of rhC1INH administered
Patient 1 31 y/o female	100	47	Asthma Eczema Rhinitis	Levocetirizine 4dd	ER - 1x Adrenalin 1x Prednisolone	2.8	1.25	0.30	275	4200 U
Patient 2 37 y/o Female	72	6	Rhinitis	Levocetirizine 4dd	ER – 1x Adrenalin 1x Clemastine Dexamethasone	3.7	0.95	0.15	163	3615 U
Patient 3 56 y/o Male	85	103	-	Levocetirizine 4dd	Prednisolone	2.6	1.19	0.27	254	4200 U
Patient 4 33 y/o Male	74	8	-	Desloratadine 4dd Clemastine 1dd	Prednisolone	15.8	1.54	0.52	98	3715 U
Patient 5 25 y/o Female	85	84	-	Levocetirizine 4dd Montelukast 1dd	-	2.8	1.70	0.34	87	4200 U
Patient 6 60 y/o Female	116	21	-	Levocetirizine 4dd	Desloratadine Clemastine	4.2	1.21	0.23	95	4200 U

Table 1. Demographic and clinical characteristics of included patients.
Legend:1 six months before start. Reference values for C1-INH function: 0.63-1.82 U/mL. C4 level: 0.1-0.47 g/L. IgE level: 0-100 kU/L.

In patient 1, the attack frequency was reduced by 85% (6.8-fold) during the treatment period compared to that during the observation period (3 versus 20 attacks, respectively; Figure 1). One of the three attacks in this patient during the second treatment month occurred 7 days after the last rhC1-INH administration when the patient had missed a treatment visit. The AAS28 scores of this patient decreased 8-fold during treatment months compared to the observation period with an accumulated AAS score of 29 in the two treatment months versus 233 in the two observational months (table 2). AE related quality of life (AE-QoL) scores improved, from 26 (small effect on AE-QoL) during the observation period to 12,5 (no effect on AE-QoL) during treatment.

		Observation 1			AAS28		Observation 2			AAS28
Patient 1	18	26	0	14	**58**	30	48	44	53	**175**
Patient 2	33	10	0	33	**76**	10	13	0	48	**71**
Patient 3	0	0	6	12	**18**	0	0	0	7	**7**
Patient 4	8	13	19	24	**64**	17	9	12	0	**38**
Patient 5	25	3	11	9	**48**	1	5	0	10	**16**
Patient 6	19	31	0	3	**53**	0	0	9	0	**9**
		Treatment 1			**AAS28**		**Treatment 2**			**AAS28**
Patient 1	8	0	0	0	**8**	12	9	0	0	**21**
Patient 2	12	15	0	0	**27**	67	35	36	0	**138**
Patient 3	8	31	0	9	**48**	9	22	0	18	**49**
Patient 4	38	19	14	0	**71**	8	4	3	2	**17**
Patient 5	3	7	16	12	**38**	0	4	6	0	**10**
Patient 6	17	7	34	22	**80**	1	0	22	13	**36**

Table 2. Angioedema Activity Score (AAS) during the observational and treatment months.
Legend: AAS7 accumulated into AAS28 are presented for the observational and treatment months.

None of the other patients (patients 2 to 6) showed a clinical response to rhC1-INH either measured as attack frequency or accumulated AAS28 scores. No improvement of AE-QoL during treatment was observed (Supplementary table 2). Patient 3 showed a trend of a higher AAS score during the treatment period. Patients 4, 5 and 6 had lower AAS scores in the second treatment and observation periods compared to those during the first treatment and observation period.

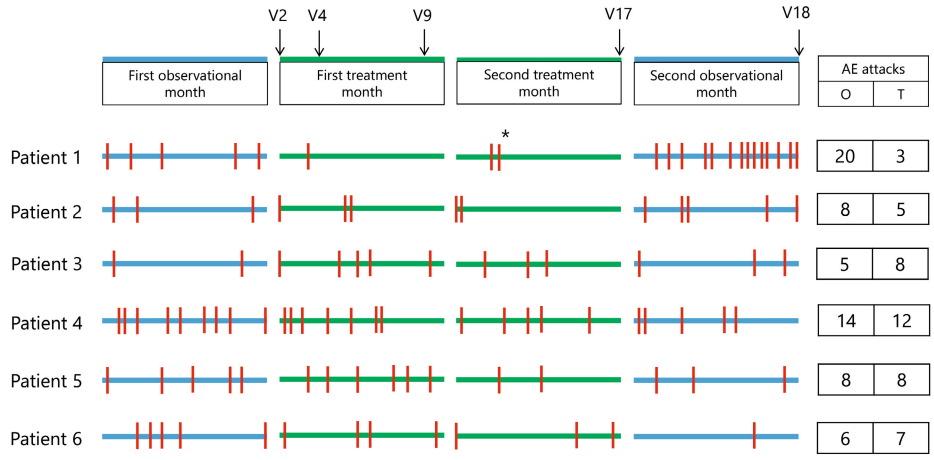

Figure 1. Four month study sequence and attack episodes per month.
Legend: trial visits at which blood is drawn (V2, V4, V9, V17, V18) are indicated with an arrow. Treatment visits occur twice a week during the treatment months in which rhC1-INH is administered. Visit 18 marks the end of the trial, no pdC1-INH was administered during this visit. One attack episode is indicated with a vertical line and could last for multiple days. Cumulative angioedema (AE) attacks in the observation months (O) versus the treatment months (T) is presented.
* AE attack occurred seven days after last rhC1-INH administration due to a missed treatment visit

No major adverse events and minimal use of escape medication during treatment were reported

Administration of rhC1-INH in patients with normal C1-INH levels did not lead to any severe adverse events, including thrombotic events. Three patients reported episodes of headache during the treatment. One patient reported an uncomplicated herpes labialis flare-up in treatment week 7. Escape medication used by patient 1 in the first observation period was one dose of 50mg prednisolone with little effect and one-time use of the adrenaline auto-injector (0.3mg). No escape medication was used during the treatment period. In the second observation period (after 8 weeks of treatment) seven doses of prednisolone were used ranging from 20 to 60 mg, with no to little effect, and four adrenaline auto-injector over two episodes were used by patient 1. Patient 3 used one dose of 20mg prednisolone during the treatment period and one during the second observation period, both with good effect. Patient 4 used one dose of 60mg prednisolone during the first observation period with good effect. The other patients did not use escape medication during the study period. No icatibant or other BK-targeting treatments were used throughout the study.

Lack of biomarkers predicting treatment response

Levels of C1-INH, HK, PK and FXII during treatment did not differ from those during the observation periods (Figure 2). These levels were all normal except for PK levels in patient 3, which were decreased but did not change during rhC1-INH treatment.

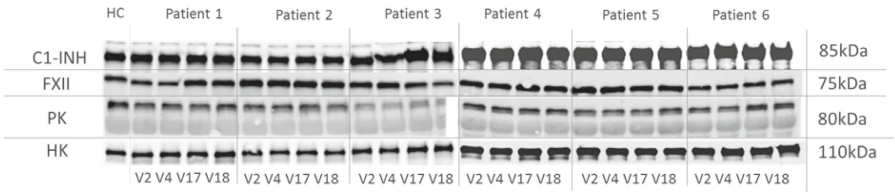

Figure 2. Proteins of the contact system.
Legend: Immunoblot of the proteins of the contact system C1-INH (C1-esterase inhibitor), HK (high molecular weight kininogen), PK (plasma kallikrein) and FXII (factor XII). Healthy Control (HC) represents a healthy pooled plasma sample. Material was taken pre- and post-treatment at visit V2 and V18 in the observation period and during treatment at visits 4 and 17(see also figure 1), with samples being collected prior to rhC1-INH administration.

All measured % cHK levels were within the normal range with little variation throughout the study in all patients (upper normal limit cHK <20%; mean levels in patient 1 15± 4 (SD)%; patient 2 3± 2%; patient 3 2± 1%; patient 4 15± 4%; patient 5 7± 1%; patient 6 4± 3%) (Supplemental Figure 2A). Mean D-dimer levels were slightly elevated compared to the reference range (<0.50 mg/L) in patient 1 (0.54 ± 0.06 mg/L), patient 3 (1.42 ± 0.15) and patient 4 (1.62 ± 0.94,), whereas they were normal in patients 2, 5 and 6. None of the patients had an attack when blood samples were collected (Supplemental Figure 2B). CRP and leukocyte counts in patients were within, or slightly above the normal range (Supplemental figure 2C&2D).

Post-trial follow-up reveals a heterogeneous treatment response

Patient 1 restarted rhC1-INH treatment after the second observation period initially with a standard treatment interval of 3-4 days achieving again rapid and complete remission (table 3). This was maintained when extending the treatment interval to 5 days. After seven months, due to health insurance limitations, treatment was switched to omalizumab 300mg/4wks and tranexamic acid 1000mg twice daily for three months, resulting in frequent and severe AE with 15 (AAS28:87) and 11 (AAS28: 101) attacks in the first two months and also in month 3, of which detailed data are missing. In this period, admission to emergency room or intensive care was required seven times. Treatment of attacks with 1000 IU pdC1INH was effective. Subsequently, health insurance approval was granted and prophylactic treatment with 1000 IU pdC1-INH every 3-4 days was initiated, which again led to immediate symptom control. After one year, treatment was switched to 4200IE rhC1-INH since herewith, remission could be achieved with longer intervals of 5-6 days. After one year, treatment was switched to 4200 IU rhC1-INH every 5-6 days, which successfully prevented the development of attacks. Additional genetic analysis supported the InH-AAE diagnosis, since no known gene mutations associated with various forms of HAE were identified. (see supplemental table 1).

Patient 2 initiated omalizumab after the study and reported partial response after six months of treatment. Consequently, the treatment dose was increased incrementally to 600mg/3wks, which resulted in a good treatment response after 28 doses.

Patient 3 wished for no further treatment additional to antihistamines and accepted symptoms with no further follow-up.

Patient 4 started post-trial tranexamic acid three times daily 500mg alongside desloratadin 5 mg four times daily, resulting in a decrease in frequency and severity.

Patient 5 started icatibant as attack medication during a two-month period. Due to ineffectiveness, icatibant was ceased and the patient recently started omalizumab treatment. The first three doses did not yet result in improvement.

Patient 6 showed an immediate near complete response after the first dose of omalizumab with an AAS28 score of 5 after the first omalizumab dose compared to a mean AAS28 score of 32 in the six weeks before omalizumab treatment.

	Post-study drug	Max dose	Total time of use in months	Effectiveness
Patient 1	pdC1INH	1000IE	12	Very effective
	rhC1INH	4200IE	19	Very effective
	Omalizumab	300mg/4wks	3	Poor
	Tranexaminic acid	3dd1000mg	3	Poor
	Prednisolone	60mg	Attack use only	Poor
Patient 2	Omalizumab	600mg/3wks	33 months	Yes, after 28 doses
	Dexamethasone	10mg	Attack use only	Yes
Patient 3	Prednisolone	20mg	Attack use only	Yes
Patient 4	Desloratadine	1dd5mg	23 months	Good, near complete response
	Tranexamic acid	3dd500mg	23 months	
Patient 5	-			
Patient 6	Omalizumab	300mg/4wks	2 months	Good, near complete response

Table 3. Real-world post-study Inh-AAE treatment.
Legend: Antihistamine use shown only for patient 4 due to the reported positive treatment response in combination with tranexamic acid.

DISCUSSION

This is the first prospective trial describing the successful use of rhC1-INH as prophylactic treatment in one out of six patients with InH-AAE (patient 1). Restart resulted in an equally fast and beneficial response. pdC1-INH also appeared to be effective. We hypothesize that the bradykinin route is pathomechanistically involved although we found no formal proof after biomarker investigations. Post-trial, patients 1, 2, 5 and 6 received omalizumab treatment. Patients 2 and 6 experienced a good, and a near complete response respectively. Patients 1 and 5 reported no clinical effect of omalizumab.

We observed an almost complete and fast improvement of AE activity in patient 1, with only 3 mild attacks in the treatment period versus 19 attacks in the observation period. Equal effectiveness was found during post-trial follow-up upon restart of rhC1-INH treatment on two different treatment periods with a similarly fast and near complete response. This was unlikely a placebo effect, since post-trial follow-up data showed a similar response to pdC1-INH, whereas treatment with omalizumab had no effect and resulted in an increased attack rate. Future research in placebo controlled studies is required to get more insight in a possible placebo effect. The effect of rhC1-INH was in line with that in HAE, in which a sustained effect of 72 hours or longer was observed in 93% of HAE patients despite the short half-life of approximately 3 hours.[149] Clinical response to recombinant (r)hC1-INH in patients with InH-AAE was not published before. Three previous case reports describe successful use of plasma derived (p)dC1-INH prophylaxis in four patients with InH-AAE.[45-47] However, from these reports, it is not clear to what extent the observed responses were due to a placebo effect, and what proportion of patients may respond to such therapy. Our data show that only a proportion of the patients with InH-AAE may respond to C1-INH treatment. Failure of efficacy of rhC1-INH in the other 5 InH-AAE patients may suggest that bradykinin is not involved in generating attacks in these patients. However, we favour another explanation. A dose of 50 IU rhC1-INH per kg increases circulating C1-INH levels by approximately 2-fold. This increase may have been too low, especially since factor XIIa bound to an activator is less well inhibited by C1-INH.[150]

None of the biomarkers of the bradykinin route (C1-INH, FXII, PK, HK) differentiated the responding patient from the others. Moreover, none of these biomarkers pointed to an increased activation of the contact system. One may argue that levels of bradykinin could provide a better biomarker for such activation. This is supported by the finding of elevated bradykinin levels in InH-AAE patients at the time of AE attacks.[48] Considering the technical issues, such as processing of plasma samples, we did not assess bradykinin levels in the patients described here.

After the study period, treatment of patient 1 with both rhC1-INH and pdC1-INH resulted in an immediate effect, though the effective treatment interval appeared to be 3-4 days for pdC1-INH versus 5-6 days for rhC1-INH. Previous studies show that there is no fundamental difference in efficacy between pdC1-INH and rhC1-INH in patients with HAE, when similar doses, expressed as IU, are given.[34] However, doses given to patient 1, who had a body weight of 100 kg, were different, 4200 IU of rhC1-INH versus 1000 IU of pdC1-INH per administration. Thus, the increase of C1-INH activity, which was approximately 1 U per ml in case of rhC1-was considerably lower, about 0.25 IU, when pdC1-INH was administered. Therefore, C1-INH levels may have decreased below a critical level more rapidly in cases of pdC1-INH administration, in spite of its longer half-life.

The restart of C1-INH treatment in patient 1 resulted in an immediate effect with a clear difference of the effective treatment interval of 3-4 days for pdC1-INH versus 5-6 days for rhC1-INH. Previous studies show that there is no fundamental difference in efficacy

between pdC1-INH and rhC1-INH in patients with HAE.[151] The observed difference in treatment interval is therefore most likely explained by dosage inequivalence since rhC1-INH is dosed at 50IU/kg with a maximum of 4200IU and pdC1-INH at a fixed dose of 1000IU.[152, 153] In our patient with body weight 100kg, 1000IU pdC1-INH might have been relatively under dosed compared to 4200IU rhC1-INH resulting in lower effectivity and the need for shorter treatment intervals.[154]

C1-INH treatment is generally well tolerated, though recent studies suggest a small risk of thrombotic events upon treatment with rhC1-INH and pdC1-INH in HAE patients.[155, 156] We did not observe thrombotic or any other adverse events in the patients studied, even though we supplemented C1-INH in patients with normal levels.

A review of omalizumab for InH-AAE in six small case series, reports a complete response in all 20 patients, with time to response ranging from one day to 16 weeks.[43] Four patients with InH-AAE who did not respond to rhC1-INH treatment, were subsequently treated with omalizumab. Two patients responded to this therapy. Patient 1 and 5 did not show any benefit from three months omalizumab treatment, although the possibility that the treatment period with omalizumab was too short cannot be excluded.[157] Prospective evaluation in an unselected population is needed to gain insight about the real percentage of responders. Response to omalizumab may be interpreted as evidence for histamine as the main mediator of AE in these patients.[157] One should be careful about making this conclusion, as marked activation of FXII, PK, and kininogen has previously been found during anaphylaxis, indicating bradykinin cannot be ruled out as the main mediator of AE even in conditions commonly associated with mast cell activation.[158]

In conclusion, rhC1-INH treatment was effective in 1 of 6 InH-AAE patients. Response to omalizumab and tranexamic acid during follow-up in some of the other patients points to a heterogeneous pathogenesis of this disease and, as a consequence, the need for a personalised treatment approach.

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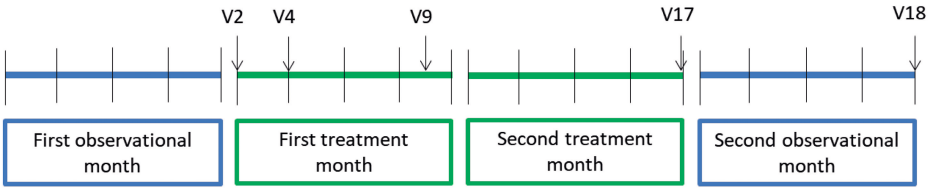
SUPPLEMENTAL TABLES AND FIGURES

A2M	F2	KLK4	PTGS1
ACE	HRH1	KLK5	PTGS2
ANGPT1	HRH3	KLK6	SERPINA1
BDKRB1	HRH4	KLK7	SERPINA4
BDKRB2	KLK1	KLK8	SERPINB2
CPB2	KLK10	KLK9	SERPINE1
CPM	KLK11	KLKB1	SERPINF2
CPN1	KLK12	KNG1	SERPING1
CPN2	KLK13	MASP1	TFPI
DPP4	KLK14	MASP2	VEGFA
F11	KLK15	PLAU	XPNPEP1
F12	KLK2	PLAUR	XPNPEP2
F13B	KLK3	PLG	MYOF
			HS3ST6

Supplemental table 1. Gene panel analysed in patient 1 (rhC1-INH responder).

	Functioning						Fatique/Mood					
	O1	O2	Final O	T1	T2	Final T	O1	O2	Final O	T1	T2	Final T
Patient 1	12	12	**8**	4	4	**0**	10	13	**6.5**	10	13	**6.5**
Patient 2	16	12	**10**	4	12	**4**	10	7	**3.5**	9	6	**2.5**
Patient 3	7	4	**1.5**	10	9	**5.5**	7	8	**2.5**	8	8	**3**
Patient 4	7	5	**2**	4	4	**0**	16	8	**7**	10	9	**4.5**
Patient 5	13	7	**6**	14	12	**9**	12	7	**4.5**	14	13	**8.5**
Patient 6	9	4	**2.5**	14	4	**5**	19	14	**11.5**	18	12	**10**
	Fear/Shame						**Nutrition**					
	O1	O2	Final O	T1	T2	Final T	O1	O2	Final O	T1	T2	Final T
Patient 1	14	14	**8**	9	12	**4.5**	5	6	**3.5**	4	3	**1.5**
Patient 2	24	18	**15**	20	23	**15.5**	7	7	**5**	2	8	**3**
Patient 3	17	16	**10.5**	15	16	**9.5**	5	5	**3**	4	5	**2.5**
Patient 4	20	15	**11.5**	12	14	**7**	4	3	**1.5**	2	2	**0**
Patient 5	24	20	**16**	23	23	**17**	2	2	**0**	2	2	**0**
Patient 6	22	19	**14.5**	26	17	**15.5**	7	5	**4**	9	3	**4**

Supplemental table 2. Angioedema quality of life score.
Legend:O1: Observational month 1, O2: Observational month, T1: Treatment month 1, T2: Treatment month 2. Minimum/Maximum scores for each domain: Functioning: 4/20. Fatique/Mood: 5/25. Fear/Shame: 6/30. Nutrition: 2/10. Final QoL scores calculated as: Entered score for the two observational or two treatment months minus twice the minimum score, divided by two.



Supplemental figure 1. Trial design with blood drawn visits.
Legend: Four month study sequence divided into weeks by vertical lines. Trial visits at which blood is drawn are indicated with an arrow. Visits occur twice a week during the treatment months in which rhC1-INH is administered. Visit 18 marks the end of the trial, no pdC1-INH was administered during this visit.

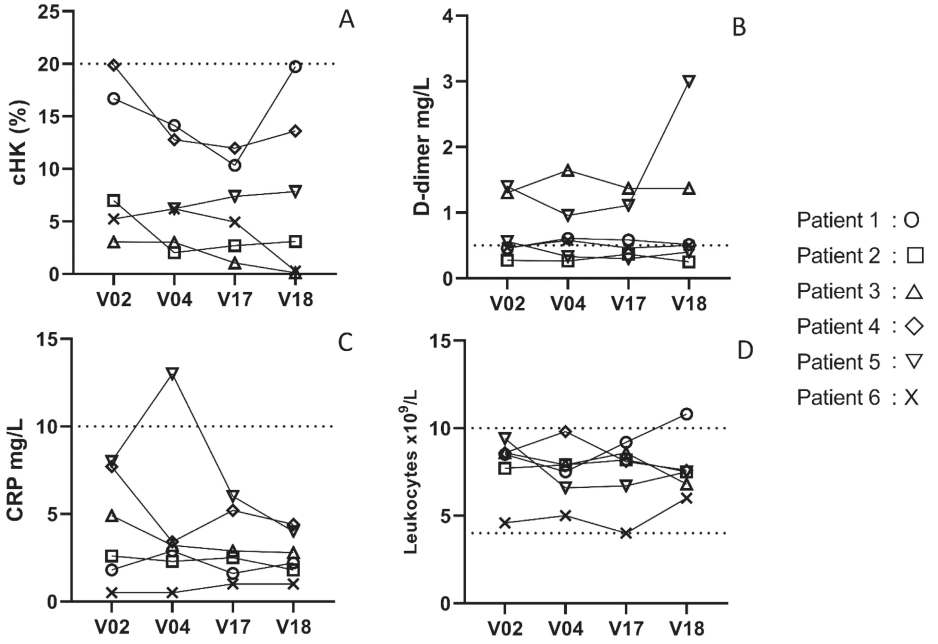


Figure S2. cHK, d-dimer, CRP and leukocyte laboratory assessment
A) % Cleaved kininogen (cHK). 100% indicates cleavage of the total HK pool. Material was taken pre- and post-treatment at visit V2 and V18 in the observation period and during treatment at visits 4 and 17(see also figure 1), with samples being collected prior to rhC1-INH administration.. Dotted lines represent upper range or normal ranges. Data V02 Patient 5 missing. Reference range <20% B) D-dimer levels in mg/L over time. Reference range 0.00-0.50 mg/L. C) CRP (mg/L) over time during study. Reference range <10mg/L. D) Leukocytes ($x10^9$/L) over time during study. Reference range 4.0-10.0 $x10^9$/L

CHAPTER 10

General discussion

In this thesis, we investigated clinical patterns and treatment improvement for patients with chronic urticaria, in search of a more personalised treatment approach. Furthermore, we aimed to improve the understanding of the therapeutic mechanisms of omalizumab and recombinant human C1-esterase inhibitor (rhC1-INH) and pathomechanistic pathways involved in CU.

MAIN FINDINGS

Part I. Optimization of omalizumab treatment in adult and paediatric patients with chronic urticaria.

Omalizumab has been approved since 2014 as add-on therapy for patients with chronic urticaria (CU) (including idiopathic angioedema (AE)) in which a fourfold dose of antihistamines is ineffective. Based on the results of randomized controlled trials, national and international guidelines recommend a standard dose of 300 mg omalizumab every four weeks. However, about one in four patients does not respond to the standard dose. In these cases, dose increase may be a good treatment option, since higher doses of omalizumab are already in use in asthma treatment and have been found to be effective and safe.

There is currently no consensus about how to adapt omalizumab treatment in patients with either complete or good response versus those with insufficient or even non-response. Treatment dose, treatment interval, treatment duration and protocol of discontinuation differ between countries and, due to lack of studies, is not addressed in international guidelines. In the first part of this general discussion, we discuss the outcomes of improving urticaria therapy, aiming to optimize the stepped-care model.

In **chapter 2,** we present the performance of omalizumab in daily practice analysed in a multicentre adult CU cohort using drug survival analysis. The overall drug survival rates for omalizumab decreased from 76% to 37% over the first 5 years, mostly determined by well-controlled disease activity. This indicates that omalizumab is discontinued in the majority of patients due to remission and not due to side-effects or ineffectiveness. This also indicates that more than a third of patients requires omalizumab treatment for more than 5 years. Patients who reported a fast response had a greater chance of discontinuing treatment due to well-controlled disease, while patients with concomitant autoimmune disease or a disease duration of two years or longer had a lower chance of discontinuing treatment due to well-controlled disease.

The performance of omalizumab in a multicentre paediatric CU population was the topic of **chapter 3**, and demonstrated comparable drug survival rates to in adult patients, with 1- and 2-year drug survival rates of 62% and 50%, respectively. Well-controlled disease activity was also the most frequent reason of discontinuation in children, indicating a similarly favourable treatment course in both adults and children. In **chapter 4,** we

demonstrate in a daily practice study that 27% of patients do not sufficiently respond to standard dose treatment (300mg per 4 weeks). Fortunately, higher doses of omalizumab (up to 450mg/600mg per 2 weeks) resulted in complete or partial response in 61% of these patients without an increase in the number or severity of side-effects.

On the other hand, we observed in patients with complete or good treatment response, that 82% could prolong treatment intervals beyond the standard interval of 4 weeks while maintaining complete or good disease control, presented in **chapter 5**. It was found that the median interval while maintaining effectiveness was seven weeks, which strongly reduces drug and healthcare costs. Patients with a fast response to treatment (<4 weeks) or with a second treatment episode (restart in cases of recurrent disease after discontinuing treatment) had a higher chance of successfully extending treatment intervals.

In **chapter 6,** we characterised the clinical profile of CSU patients with angioedema (AE) as main presenting symtom. We focussed on idiopathic AE with and without wheals, in terms of attack frequency and disease control related to treatment response**.** No prophylactic treatment was used in a substantial part of our population (27%), of which the majority showed no attack during follow-up, which might suggest that the natural course of the disease can be relatively mild. This was also supported by our finding that 81% of this group did not use any treatment during an attack.

Prophylactic antihistamine monotherapy, the preferred first line treatment, was used in 59% of patients and resulted in well-controlled disease in 77%. Add-on treatment, mostly omalizumab, was prescribed in 14% of patients. Difficult to treat disease was observed in 8% with no response to a four-fold dose of antihistamines and/or omalizumab. These findings were similar in patients with idiopathic AE with or without wheals.

Part II. Exploring the pathomechanistic pathways involved in CU and working mechanism of omalizumab and recombinant human C1-esterase inhibitor (rhC1-INH).

To date, the exact pathogenesis of CU including AE and the complete mechanism of action of omalizumab in chronic urticaria remain unknown. We conducted a prospective study with 30 patients with severe CSU to investigate the clinical effectiveness in relation to the mechanism(s) of action of omalizumab and reported the results in **chapters 7 and 8**. Fifty percent of patient (n=15) showed complete or good treatment response after six months of treatment, 20% (n=6) showed this clinical response already within the first month of treatment, indicating a fast response of omalizumab in a proportion of the patients. In **chapter 7,** the potential involvement of the complement system in pathogenesis of CU was examined in serum and skin biopsies of all included patients at baseline and during omalizumab treatment. Lesional skin biopsies at baseline revealed complement deposition (C4d) in blood vessels in the papillary dermis of 53% (16/30) of the patients, indicating local complement- activation in a significant percentage of CSU patients. In addition, increased C5a levels in peripheral blood were found at baseline

compared to healthy controls, which suggests complement activation in active CSU. However clinical effectiveness of omalizumab could not be predicted based on baseline findings or changes in complement components over time.

Mast cells are considered the most important effector cell in CSU, although other FcεRI bearing leukocytes have been suggested also to be involved. In **chapter 8,** we described the responsiveness and FcεRI expression of basophils, eosinophils, monocytes, and three types of dendritic cells (pDCs, mDC CDC141+, and mDC CD1c+) in patients with CSU treated with omalizumab. Surprisingly, we found that FcεRI expression on basophils decreased significantly within as few as seven days after omalizumab administration, while anti-IgE-induced degranulation increased significantly. These effects persisted for at least 3 months after treatment discontinuation. None of these findings however, could predict treatment response, since no significant differences between responders and non-responders were found. The decreased FcεRI expression after omalizumab was also detected on the mDC CD1c subset and pDCs, but not on monocytes nor on the mDC CDD141 subset and could likewise not predict therapy response. The clinical relevance of these findings therefore warrants further investigation. In AE, two main pathways are thought to mediate the typical acute swellings, namely, the histamine pathway and the bradykinin pathway. Patients without proven C1 esterase inhibitor deficiency (HAE/AAE) that do not respond to antihistamine therapy and for which no underlying cause can be found, such as drug hypersensitivity, ACE-inhibitor use or genetic defects associated with bradykinergic AE, are considered as having idiopathic AE. In patients with idiopathic AE who are unresponsive to antihistamine treatment (idiopathic non-histaminergic AE (InH-AAE)), add-on treatment with omalizumab is initiated in agreement with CU guidelines. In hereditary angioedema, treatment is supplemented with C1-esterase inhibitor (C1-INH) in order to inhibit coagulation factor XIIa and plasma kallikrein (PKal), preventing excess generation of bradykinin and formation induction of angioedema. As described in **chapter 6**, 44% of patients with idiopathic AE did not respond to omalizumab treatment, which has a tendency to be higher than for CU (27%). We hypothesised that supplementing C1-esterase inhibitor, that is a very effective treatment in hereditary angioedema, might be effective in antihistamine and omalizumab nonresponsive patients with idiopathic AE also. Therefore, we prospectively investigated the effect of rhC1-INH prophylaxis in patients with InH-AAE, and searched for the pathomechanism, as presented in **chapter 9**. One out of six patients showed a strong reduction in attack frequency of 85%, and also a good response to plasma-derived C1-INH, but not to omalizumab. Unfortunately, none of the measured biomarkers at baseline and during treatment could be related to treatment response. Of the four patients who did not respond to rhC1-INH and were post-trial treated with omalizumab, two showed good clinical effectiveness, indicating different pathomechanisms in AE patients with the same disease phenotype.

DISCUSSION OF THE CHAPTERS

In daily practice omalizumab is highly effective and safe in patients with CSU

Omalizumab treatment is effective and safe

In **chapters 2 and 3,** we analysed the performance of omalizumab in daily practice of an adult and paediatric population, respectively. In our large adult CU population (n=659), 39% reported complete response, 41% good response, 17% partial response and 4% poor response. This corresponds well with previous real word studies which report treatment effectiveness in 85% of patients of which 57% report complete response.[1] Traditional methods of reporting treatment effectiveness however, address outcomes such as therapy response and side-effects separately and do not consider these factors simultaneously. By using drug survival analysis, we were able to measure the performance of a drug in daily practice, which incorporates both effectiveness and safety, as well as other factors including preferences of both patients and doctors. We found that the median overall survival rate of omalizumab was 2.7 years and that the overall drug survival rates decreased from 76% to 37% over the first 5 years. Omalizumab drug survival was mostly determined by well-controlled disease which was the reason for treatment discontinuation in 50% of patients after the first 5 years of treatment.

Ineffectiveness and side-effects were only accountable for treatment discontinuation in a small portion of patients: 12% and 5% after the first 5 years respectively. This indicates that that a much smaller percentage of patients discontinue treatment due to ineffectiveness or side-effects, confirming both effectiveness and safety. These results might aid patients and physicians in managing their expectations of omalizumab treatment. Furthermore, it is likely that these results are influenced by the fact that at present, omalizumab is the only add on treatment option, which might change in the future, which would subsequently impact omalizumab's drug survival - as new options become available, treatment decisions might change and omalizumab may be discontinued sooner in favour of another drug.

Three previous studies reported overall drug survival rates of omalizumab in an adult population and confirmed high effectiveness and safety, but did not report drug survival rates or determinants of drug survival for each major reason of discontinuation.[2-4] Our group previously reported overall omalizumab drug survival rates of 77%, 61% and 55% for the first three years, respectively in an adult population that was in part similar to our current study[2-4]. In our previous study involving 142 subjects, we identified chronic inducible urticaria (CindU) as a determinant for increased drug survival related to well-controlled disease activity. Although these 142 patients were also included in our current study, this finding could not be reproduced in our recent study. Ghazanfar et al. found an overall omalizumab drug survival rate of 78% for the first half year, which

is lower compared to the 95% for the first half year in our study population (data not shown). Ke et al found a more comparable overall drug survival rate of at least 72% after one year and 60% after 18 months. Factors including the local treatment protocol and reimbursement may explain the difference in overall drug survival, which highly influence physician decisions

In our omalizumab-treated paediatric population, aged 3 to 17 years, **(chapter 3),** we found a similar high treatment effectiveness with 40% reporting complete and 37% good response when compared to the adult population. Overall drug survival rates were 62% and 50% for the first and second year respectively, comparable to that in adults (p=0.220, data not shown), and also mostly determined by well-controlled disease. Due to the small sample size, no analysis for predictors of drug survival could be performed in the paediatric population. No omalizumab drug survival studies have previously been performed in a paediatric population. Two recent daily practice studies in children (<17 years), with small patient numbers (n=12 and 19) reported similar complete response rates of 42%[5] and 68%[6]. Information regarding omalizumab response in children of <6 years with CU is very scarce with only reports on nine patients. In our population, side-effects were reported in 32% of patients, but were generally mild and transient (discussed below).

When analysing which factors influence the different reasons for treatment discontinuation for our adult population (determinants of drug survival), we found that fast response was a predictor of a shorter treatment course while a longer disease duration before start of omalizumab was a determinant for longer treatment before discontinuation due to well-controlled disease.

Timing and reason for treatment discontinuation is predictable by a number of specific determinants.
In the adult population discussed in **chapter 2,** fast response (in case of complete or good response) was observed in 41% of our adult population and was associated with a higher chance of discontinuing treatment due to well-controlled disease. This supports the hypothesis that in fast responders, the FcεRI pathway is activated through IgE crosslinkage by an autoallergen (type I autoimmunity) resulting in chronic urticaria symptoms, which is rapidly and effectively blocked by omalizumab.[7] In slow-responders, the clinical urticaria symptoms are probably caused by type IIb autoimmunity and the effect of omalizumab is thought to be due to an indirect mechanism, i.e. internalisation of FcεRI, thus preventing IgG autoantibodies from binding to unoccupied FcεRI or crosslinking IgE bound to FcεRI, which perhaps requires more time.[8] Autoimmune diseases have previously been reported in up to 28% of cases and are linked to type IIb autoimmune CSU, in which IgG autoantibodies are believed to directly activate mast cells by binding to FcεRI.[8, 9]

These autoimmune diseases include Graves' disease, Hashimoto's hypothyroidism, Sjögren's syndrome and diabetes type I. Furthermore, IgG autoantibodies against thyroid peroxidase (TPO) have been found in up to 54% of patients with CSU.[10] Recent studies introduce the idea of "overlapping autoimmune diseases" which states that that disorders which are autoimmune in nature occur at increased frequency in patients with known autoimmune disease.[8, 11] Omalizumab, which binds selectively to IgE and not IgG, was found to induce slower treatment response in patients with detectable serum autoantibodies directed against either the cell-bound IgE or unoccupied FcεRI.[12] Due to the retrospective nature of our study however, autoimmune disease status was limited to the available data in the patients' electronic health record, and might not reflect the patients' complete and actual autoimmune disease status.

Discontinuing omalizumab treatment due to side-effects is rare

Side-effects of omalizumab have previously been reported as mild and transient.[1, 13] We found similar results in our studies (as discussed in **chapters 2 and 3),** with the most reported side-effects being equal for both the adult and paediatric populations - fatigue, headache and flu-like symptoms. It is currently unknown as to what extend the occurrence of side-effects would result in treatment discontinuation. By means of drug-survival, we found that although a high percentage (72%) of adults reported side-effects, discontinuing treatment due to side effects was rare, applying to only 4% of patients. A similar effect was seen in our paediatric population with 32% reporting side-effects during omalizumab treatment and only 3% discontinuations due to side-effects. Safety data in the treatment of paediatric patients were previously not reported. [14, 15] Only a small number of case reports with six patients reported safe and effective use of omalizumab in children and adolescents.[16] In asthma, a meta-analysis with large patient numbers (n=1380) investigated the safety profile of omalizumab in paediatric patients. They reported a slightly higher percentage of side effects of 5% compared to placebo. In line with our findings, no paediatric patient discontinued omalizumab due to side effects.[17, 18] The side effects reported most in omalizumab-treated children with asthma are nasopharyngitis, upper respiratory tract infections and gastrointestinal disorders. This is considerably different from that in our CU population and might be explained by the fact that sinopulmonary infections are more common in children with severe asthma.[19] However, in both diseases they were mild and mostly transient.

Taken together, the very small percentage of patients discontinuing omalizumab treatment due to side effects confirms the high safety of the drug. However, these data are probably also influenced by the fact that, at the moment, no viable alternative treatment is available, which might stimulate patients to accept side-effects when experiencing good effectiveness, which subsequently contributes to longer drug survival. Possible further expansion of therapeutic options will probably influence side-effect related drug survival.

Improving effectiveness by increasing the omalizumab dose

Omalizumab should be updosed in patients with CU not responding to the standard dose

Current international guidelines recommend omalizumab treatment at the approved dose of 300mg every 4 weeks in adults and adolescents with CSU.[20] However, in our daily practice study (**Chapter 4)** a considerable proportion (27%) of patients did not sufficiently respond to standard doses of omalizumab. This corresponds to earlier daily practice studies which report 12-30% of patients not responding to standard doses of omalizumab.[21, 22] Increasing the treatment dose in our patients who did not sufficiently respond to the standard dose was effective in the majority of patients: 61%, of which 32% showed a complete and 30% a partial response. Overall, this is comparable to the studies of Vadasz and Curto-Barredo.[21, 22] We found that no additional improvement was derived from shortening the treatment intervals to two weeks, although the number of patients was very small (n=9). No other studies have investigated this to date.

Our data contrasts with the first phase II dose-finding study (n=90) in which 300 mg and 600 mg per 4 weeks were compared and no clear benefit in treatment effectiveness (measured by UAS7 scores) of 600mg in comparison to 300mg was observed.[23] This study was however not designed to investigate the additional effect of 600mg omalizumab in patients not responding to 300mg. Furthermore, this dose finding study was never repeated in a larger cohort.

Recently, in a review by Metz et al. on the effectiveness of high dose omalizumab in patients who failed to reach sufficient treatment response on standard doses, updosing appeared effective in more than half of the patients.[24] There was a variation between the studies in patient numbers (n=2 to n=286) and treatment doses (450/4wks to 600mg/2weeks). This review describes a total of 1207 patients with complete response rates due to updosing omalizumab of 32%-75%.

Altogether, there is substantial evidence that updosing up to 600mg is effective and should be considered for patients not responding to standard doses of omalizumab. This is even more important since the other currently available treatment options, cyclosporine and prednisone, are associated with considerably more side-effects. It is important to point out and inform patients that updosing omalizumab, as well as cyclosporine use for CSU are currently off-label, but they are supported by national and international guidelines.

In patients with moderate to severe allergic asthma, the dosage of omalizumab is based on total IgE level and bodyweight, with treatment dosages varying from 75 mg/ 4 week to a maximum of 600mg / 2 weeks. Patients with high total IgE levels and/or high body weight are treated with higher doses of omalizumab.[25] The opposite was found in CSU , as patients with higher levels of IgE were more likely to respond to standard dose (300mg / 4 weeks) of omalizumab, in contrast to CSU patients with lower IgE levels, who

more frequently required higher doses of omalizumab.[26] This finding might suggest that the treatment effect of omalizumab relies on different mechanisms in allergic asthma and CSU for which the required treatment dosage differs.

Can the need for higher doses of omalizumab be predicted?
Currently, CU inclusive idiopathic angioedema is generally treated for at least six months with standard doses of omalizumab before treatment is updosed in patients showing insufficient response. If a favourable response to high dose could be predicted, this could be started earlier, reducing the burden of extended disease.

In our study, as described in **chapter 4**, we assessed whether age, sex, disease duration and therapy response to standard dose could differentiate between patients with response and without response to higher doses of omalizumab and whether it could be possible to use these variables to predict therapy response. None of these characteristics differed significantly between patients requiring standard and high doses of omalizumab. However, when comparing the same characteristics between responders and non-responders of high dose treatment, a trend for older patients to better-respond to higher doses was found (median 41 vs 35 respectively, p=0.080, data not shown), which might point towards a possible predictor of more effective treatment at higher-than-standard dose. Only one other study (n=286) has investigated factors predicting response to higher doses (max 600mg/4wks) of omalizumab. It found that both age >57 years and high BMI >30 as positive predictors.[22] However, this study did not investigate the correlation between age and BMI to exclude co-correlation. There, is evidence that older age is related to higher BMI for patients with CU.[27] Therefore the predictive value of BMI and age as unique variables in their own right needs further investigation. Since patient-data regarding BMI was not obtained in our study (**Chapter 4)**, it was not possible to investigate the role of bodyweight in relation to response to high dose omalizumab. Recently, several studies have suggested that a high BMI might be a predictor for nonresponse to standard doses of omalizumab in patients with CSU.[27-29] Unlike in asthma, there is no dose correction for BMI in CU patients and it may therefore be that in CU treatment, patients with a high BMI and non-response to standard doses are relatively under-dosed. This might explain why a substantial percentage of patients (**Chapter 4**) show a favourable response to updosing. Further research is required to determine whether BMI or age, or both could be used as parameters for dose adaption.

Effectiveness of omalizumab after decreasing the dose by interval extension

Omalizumab treatment intervals can be extended in the majority of patients.
In **chapter 5,** we reported that in our population, treatment interval extension to an interval of 6 weeks or longer while maintaining disease control was possible in 73% of patients, Furthermore, we found that 57% were even able to extend the interval

to 8 weeks or longer. A few retrospective studies with small patient numbers (range n= 19-49), have described successful interval extension while maintaining disease control.[30-32] Some of these patients were able to discontinue treatment. Data regarding effective treatment extension for patients with underlying active CU, i.e. patients who cannot stop treatment due to reoccurrence of symptoms, is limited to five small studies (ranging from 5 to 12 weeks, min n= 7; max n=51) .[30, 33-36] Three of these studies reported successful treatment interval extension beyond 6 weeks in 43%-80%[33-35] and beyond 8 weeks in 30%[33] of patients with active CU. In our larger population (n=84 with active CU) we found similar results, underlining the possibility of extending treatment intervals in patients with good treatment response. In addition to reducing drug exposure and potential side-effects, extending treatment intervals furthermore has an important benefit of decreasing healthcare costs.[37] In the total population of 132 patients in the study discussed in **chapter 5**, a total of 668 administrations (16%) have been saved by implementing interval extensions. Based on the 2019 single-administration cost of €760, this result if a cost reduction of €3,848 per patient . This calculation does not take into account the costs of hospital visits, concomitant medication use and the absence at place of employment due to CSU, which would further increase the cost reductions due to interval extension.

Recent studies suggest a protocol comparable to the protocol we used for successful interval extension and omalizumab discontinuation.[30, 32-35] To date, there is no clear consensus on the maximal treatment interval after which CU is considered to be in remission. However, the previously mentioned studies discontinued treatment after an interval of 8 weeks.[30, 33] In our protocol, treatment intervals of patients with well-controlled disease (UCT ≥ 12 and UAS7 ≤6) for at least 6 months, were gradually increased by 1 week and in cases of continuous and complete disease control, treatment was discontinued at an 8-week treatment interval. Clinical trial data however, show that omalizumab has a positive effect on itch and wheals over placebo up to 9 and 11 weeks after the last dose respectively, which suggests a disease free interval of 11 weeks before treatment can be successfully discontinued.

Fast responders were more likely to successfully extend treatment intervals.
The optimal timing of starting interval extension remains to be investigated since previous studies used a wide range in the initial treatment period after which interval extension was initiated (3 months[30] – 12 months[34]). It is unclear if patients could start extending treatment intervals already after the first dose in case of fast and complete response, instead of after three to six months. Since skin FcεRI bearing cells have been found to require up to 12 weeks of omalizumab treatment before showing significant downregulation of FcεRI, omalizumab treatment might require this time period to express its full effectiveness.. [38] To date, no other studies have investigated if and when patients can extend treatment intervals. Since we found that patients with a fast response to treatment were more likely to successfully extend treatment intervals,

these patients could probably extend treatment intervals sooner than 6 months. Additionally, Niemeyer-van der Kolk et al. successfully initiated extending treatment intervals after three months of treatment, further supporting the possibility of a more rapid interval extension than 6 months.[30]

A proportion of the patients remain completely symptom-free upon interval extension, implying a possible remission of disease and opportunity for treatment discontinuation. Gradual interval prolongation seems to be the preferred method towards treatment discontinuation, since it limits the possibility of a sudden major exacerbation. We demonstrated that 48% of patients could discontinue treatment after interval extension. One other study found similar results and reported that 42% of patients could increase the interval between omalizumab injections up to 8 or 9 weeks, followed by discontinuation for at least 3 months.[31]

AE treatment response is independent of the presence or absence of concomitant wheals.

The presence of wheals has no influence on the response to treatment of idiopathic AE
In **chapter 6** we evaluated the disease severity profile in patients with idiopathic AE in relation to prophylactic treatment and the presence or absence of concomitant wheals. We reported that no prophylactic treatment was prescribed in 27% (64/236) of patients with well-controlled disease reported in 86% (25/29) of these patients. Antihistamine monotherapy was used in 59% of patients and resulted in well-controlled disease in 77% of these patients. This indicates that for the majority of patients with idiopathic AE, up to fourfold the standard dose of antihistamine treatment is sufficient. Previous studies have estimated the treatment effect of antihistamine monotherapy for patients with mainly CU at 59%[39]-75%[40], of which the majority of patients (85%)[39] used antihistamines prophylactically. Similar effectiveness of antihistamine therapy in patients with idiopathic angioedema has previously been demonstrated by our group, with 64% (n=50) showing a reduction in angioedema attacks and 25% reporting complete response.[41] It is likely that the number of patients that respond to antihistamine monotherapy is even higher than reported since antihistamines are available over the counter or are prescribed by general practitioners and are not referred to the hospital.

A relatively small, but substantial proportion of patients with idiopathic AE required add-on treatment with omalizumab (9%), of which 56% was well-controlled without attacks or with low attack frequency. This percentage is somewhat lower when compared to the omalizumab response in patients with mainly wheals, for which average complete response rates of 76% have been reported.[42] The reason for this is unknown. We previously reported in a small review of 19 patients in six articles that omalizumab resulted in a complete response in 63% of patients with AE without wheals.[43] Recently, a prospective study found a higher complete response rate to omalizumab treatment in four out of five patients with InH-AAE within 24 weeks of treatment.[44] Together, the

data indicate the effectiveness of omalizumab in patients with idiopathic AE, but also underlines that a substantial part of the patients still experience symptoms despite treatment.

Blood circulating basophils and the complement system are involved in the pathogenesis of CSU, but cannot predict for the clinical response to omalizumab.

Complement factors C4d and C5a are involved in the pathogenesis of CSU
Generally skin mast cells are considered the main effector cells in CSU. Removing free IgE and subsequent internalisation of the FcεRI by omalizumab is thought to inhibit mast cell activation. While it could take up 4 to 12 weeks for skin mast cells to decrease FcεRI, the clinical effect of omalizumab is observed within days to weeks in about half of patients.[38, 45] We therefore investigated other pathways that could explain the rapid clinical effect of omalizumab. In **chapter 7** the potential involvement of the complement system in the pathomechanism of CSU and the therapeutic mechanism of omalizumab was examined. The involvement of complement in the pathogenesis of urticaria has often been postulated, but its role has not been elucidated.[46, 47] Lesional skin biopsies at baseline revealed C4d deposition in blood vessels in the papillary dermis of 53% (16/30) of patients. Furthermore, median C5a levels in peripheral blood were increased in CSU patients compared to healthy controls (1847,6 vs 959.2 pg/ml respectively). The activation of complement factors in plasma suggests complement activation in CSU, although the activation seems to be selective since plasma levels of C1q, C3, C3bc/C3, C4, C4bc/C4 and MAC were unchanged. Increased plasma C5a levels in patients with CU were previously found by Zhu et al.[48] The authors state that generation of a combination of IgG, thrombin and complement system activation are involved in the pathogenesis of urticaria.

There is evidence that besides immunoglobulins, thrombin (FIIa) is also capable of activating the complement system via the blood coagulation pathway.[49-51] In their recent review, Yanase et al. introduced the idea that tissue factor (TF) expressed on vascular endothelial cells can activate coagulation factors including FXa and FIIa which induce plasma leakage via vascular endothelial cells, allowing C5a to leak from blood vessels and activate skin mast cells.[47] C5a is known to cause chemotaxis, local inflammation and endothelial activation and might therefore induce local C4d deposition in affected skin.[52] The role of C4d is not known in CU and angioedema, but has previously been associated with vascular events, as seen during thromboembolism[53], rheumatoid arthritis[54] and SLE[55]. However, the increased C5a levels in peripheral blood of CSU patients might offer new insights into the pathogenesis of urticaria and angioedema and might offer a future target for new treatments. The blockage of the C5a receptor appeared an effective therapy in microscopic polyangiitis and granulomatosis with polyangiitis.[56] IgG, which is suggested to be involved in Type IIb CSU, can activate the complement system via the classical pathway and thus induce C5a formation. The importance of

C5a is further accentuated by the finding that basophils express both C3a and C5a receptors, but mainly C5a could induce degranulation of basophils.[51, 57] Additionally, both the complement pathway and the coagulation pathway might be linked to histamine release through basophil and mast cell activation, since C5 could not induce histamine release by itself, but needed to be activated by coagulation factors IIa and Xa to C5a. The exact relevance of increased plasma levels of C5a remains therefore to be elucidated. In our study, the clinical effectiveness of omalizumab could not be linked to plasma C5a levels or any of the studied complement components (C1q, C3, C3bc/C3, C4, C4bc/C4, C5a, and MAC). One limitation of the study was that, due to the explorative nature, we did not adjust blood C5a levels in relation to parent component protein C5. In lupus nephritis[58] and HAE[59] the ratio between an activation product and its parent complement protein was found to discriminate between active and inactive disease and might indicate systemic complement activation rather than the activation product itself. On the other hand a recent study demonstrated that complement protein MAC (sC5b-9) in relation to its parent complement C5 did not improve the sensitivity of complement activation systemically. Future studies using different approaches might therefore be required to elucidate the role of C5a in CSU, taking into account C5a levels in relation to its parent component C5.

Basophils FcεRI expression is reduced during omalizumab treatment, irrespective of treatment response.

Circulating blood basophils have previously been found to increase in response to treatment with omalizumab, possibly due to the interruption of basophils migration from the circulation into the skin.[60, 61] These fast acting FcεRI bearing leukocytes might therefore serve as a predictor for clinical response to omalizumab.[60] In **chapter 8** we describe that FcεRI expression on basophils decreased significantly within one week after omalizumab initiation, which might explain the fast clinical effect of omalizumab due to a diminished number of stimulable receptors. This decrease in FcεRI expression on basophil, was however irrespective of clinical treatment response and could not differentiate between treatment responders and non-responders. Furthermore, despite the diminished expression of FcεRI on basophils due to omalizumab, the mean in vitro basophil degranulation was significantly increased after anti-IgE–induced basophil activation, which implies that the degree of FcεRI expression alone does not determine the level of degranulation per se.

Three other recent studies investigated whether baseline basophil FcεRI expression could predict omalizumab response and found contrasting results, with one study finding a clear difference between responders and non-responders.[62-64] Deza et al. (2017)[62] found a statistically significant higher baseline level of basophil FcεRI expression in omalizumab responders and a greater percentage of reduction in FcεRI expression during treatment in responders compared to non-responders. The studies by Johal et al (2021) and Oda et al. (2021) however found no relation between baseline basophil

FcεRI expression and clinical response, which is in line with our study. Demographics and methods in the four studies seem comparable overall, and can therefore not be an explanation for the difference. The design of these four studies is comparable and the only difference is the adopted definition of treatment response. While we defined treatment response as a UAS ≤ 6 or UCT ≥ 12, the definition applied by Deza et al. was an UAS7 ≤ 6 or ≥ 90% reduction in the UAS7 and Oda et al. defined response as a UCT score of ≥12.[62, 63] Johal et al. did not define treatment response but correlated the change in UAS7 to FcεRI expression on basophils.[64] When reanalysing our data using the same definition for treatment response as these three studies, we still did not observe a difference in basophil FcεRI expression at baseline between responders and non-responders.

Also low total IgE has recently been reported as a possible predictor for omalizumab non-response in patients with CSU. It has been suggested that lower IgE levels are indicative of an autoimmune driven CU endotype, which is also associated with poor response to omalizumab.[26, 65, 66] Since there is a wide range in total IgE values amongst CU patients and no clear cut-off values for predicting therapy response are currently reported, the clinical relevance of total IgE as predictor for therapy response is questionable. However, we found in our study (**chapter 8**) no difference in total IgE levels between omalizumab responders and non-responders. Recent studies suggest measuring total IgE levels in relation to IgG anti-thyroid peroxidase (anti-TPO) as a predictor for therapy response, since a combination of high anti-TPO and low IgE have been shown to be associated with nonresponse to omalizumab in two studies.[66, 67]

An alternative treatment for omalizumab nonresponders in InH-AAE

In **chapter 9** we focussed on the treatment of idiopathic AE of patients without wheals and without response to antihistamine treatment. In AE, two main pathways are postulated to mediate acute swellings, namely, the histamine pathway and the bradykinin pathway. In patients with HAE, AE is caused by an excess of bradykinin formation, usually through the absence of (functional) C1-INH which leads to a disrupted inhibition of Factor XII and plasma kallikrein.[68] Binding of bradykinin to mainly the bradykinin 2 receptor causes vascular leakage and consequently angioedema. By supplementing C1-INH, AE is resolved in HAE patients. In InH-AAE, it is unclear which mechanism is responsible for AE formation. In **chapter 9** we reported that one out of six patients with InH-AAE showed a reduction in attack frequency of 85% during rhC1-INH treatment. A similar response in this patient was observed following plasma-derived C1-INH treatment, but not to treatment with omalizumab, strongly indicating a role of the bradykinin pathway in this InH-AAE patient.

In general, AE without other underlying causes is generally considered histamine mediated.[69] However, it is shown by Hofman et al. and our study in **chapter 6,** that a substantial proportion of these patients do not respond to antihistamines (InH-AAE).[41]

One theory is that even mast cell mediated AE is also in part bradykinin mediated, since mast cells have previously been found to be able to activate Factor XII by the release of heparin.[70, 71] Furthermore, one study found a significantly higher expression of bradykinin receptor 1 on lymphocytes and bradykinin receptor 2 on monocytes in CSU patients compared to healthy controls.[72] We therefore hypothesise that treatment with C1-INH or omalizumab, which inhibit parts of different pathways, might both subsequently affect one common pathway and therefore can be effective in the treatment of angioedema. Furthermore, it has been shown that FXIIa bound to endothelial cells is protected from inactivation by C1-INH.[73] The unresponsiveness to C1-INH treatment in other patients might be explained by a C1-INH independent pathomechanism in these patients, but also by a sub-effective dose, meaning that higher doses, that are usually used in HAE, would be needed to inactivate FXIIa. Another possible mechanism is that InH-AAE in rare cases is in fact HAE with normal C1-INH. For the C1-INH responding patient, described in **chapter 9**, we found no clues in family history or genomic analysis to indicate HAE due to genetic abnormalities. HAE-nC1-INH due to an undiscovered mutation cannot be completely excluded. Abnormalities in genes coding for Kininogen 1 (KNG1) have only recently been found to cause AE, and more undiscovered genes might follow.[74]

To date, there is no clear treatment strategy for patients with InH-AAE in the event of failure of omalizumab therapy. pdC1-INH has previously been proposed as a possible treatment for such patients, with three case reports describing successful use of pdC1-INH prophylaxis in four patients with InH-AAE, two with complete and two with partial response.[75-77] A review of Bucher et al. summarises the effectiveness of omalizumab for idiopathic AE in six case reports, with a complete response in all 20 patients.[75]

Also in our study describing the profile of symptoms in relation to treatment, 56% of AE patients reported response to omalizumab, irrespective of the presence of subordinate wheals (**chapter 6**). Furthermore, we found that two out of four patients treated with omalizumab and not responding to rhC1-INH, did respond to omalizumab (**chapter 9)**. We furthermore presented one patient who did not respond to rhC1-INH treatment but did respond to a combination of antihistamine and tranexamic acid.

Bucher et al. also investigated the use of tranexamic acid, often used in HAE, in patients with InH-AAE and reported complete response in 28% (n=27) of patients.[75] A recent retrospective study found that 69% of patients (n=20) reported significant reduction in frequency and severity of symptoms due to treatment with tranexamine acid, with 17% (n=5) reporting a complete response.[78] These studies point towards a positive effect of tranexamic acid in InH-AAE. Current guidelines offer limited treatment options for InH-AAE patients as most guidelines focus on HAE or CSU.[20, 79, 80] Based on our findings and current literature, rhC1-INH, and/or tranexamic acid treatment should be considered as add-on treatment in patients with InH-AAE, not responding to omalizumab. Since

tranexamine acid is characterized by a good safety profile and low costs, this drug could be used as a next-treatment step when antihistamines fail. However, further studies are needed to prove the efficiency of these drugs.

Future therapies of chronic urticaria and angioedema.
Various older and new therapies have been recently suggested to be (partly) effective in CSU treatment.[81, 82] Response to monotherapy of cyclosporine has previously been reported in up to 62% of patients.[83] A recent study demonstrated that concomitant treatment with standard doses of omalizumab and 1.1 – 3.7mg/kg cyclosporine offers good treatment response in up to 76% of patients who were unresponsive to both omalizumab and cyclosporine monotherapy.[82]

Drugs targeting sites other than the histamine- or IgE-receptor, that are currently being developed for other various indications are also under investigation in clinical trials focusing on CSU include benralizumab (anti-IL5Rα), bruton tyrosine kinase (BTK), CDX-0159 (anti-c-KIT receptor), dupilumab (anti-IL-4Rα), interleukin-5, Siglec-8 and tezepelumab (anti-TSLP mAb).[84, 85] A possible promising treatment strategy for patients assumed to suffer from autoimmune CU for which anti-IgE therapy seems less effective are BTK-inhibitors including remibrutinib, rilzabrutinib and fenebrutinib, which target the intracellular downstream signalling pathway of FcεRI.[85] Further knowledge of current treatment options and the development of new therapies might eventually lead to more effective and personalised treatment approaches.

Concluding remarks:
- The overall drug survival rates for omalizumab decreased from 76% to 37% over the first 5 years
- Fast omalizumab response resulted in a higher chance of shorter treatment duration with well-controlled disease and a lower chance of discontinuing treatment, due to side effects.
- A shorter disease duration before starting omalizumab led also to a higher chance of shorter treatment duration with well-controlled disease and a lower chance of discontinuing treatment, due to side effects.
- The use of immunosuppressive drugs at the start of omalizumab treatment was associated with a higher chance of ineffectiveness.
- Ineffectiveness or side effects are rare reasons for discontinuation, demonstrating the high efficiency and safety of omalizumab in the treatment of CU in daily practice.
- Omalizumab treatment intervals can be extended from the standard of 4 weeks to 7 weeks (median) or longer in patients with good/complete response.
- Treatment discontinuation might be attempted when an eight week interval has been reached without any recurrent disease.
- In patients with insufficient treatment response to omalizumab standard doses of 300mg, increase of the dose up to 600mg led to significant improvement in 61% of patients.

- Complement component C4d skin deposition and elevated blood levels of C5a indicate the involvement of the complement system in pathogenesis of CU.
- FcεRI expression on basophils decreased significantly after omalizumab administration, while anti-IgE-induced degranulation increased significantly.
- None of the findings regarding the complement system or FcεRI expression were related to omalizumab treatment response.
- Angioedema is highly manageable in the majority of patients without prophylactic therapy or antihistamine monotherapy, but 14 % required add-on treatment with omalizumab or other drugs.
- In patients with InH-AAE, (rh)C1-INH treatment might be a treatment option when omalizumab has failed.

This thesis offers new insights into pathogenesis and the management of urticaria including angioedema and suggests possibilities for a personalised treatment approach.

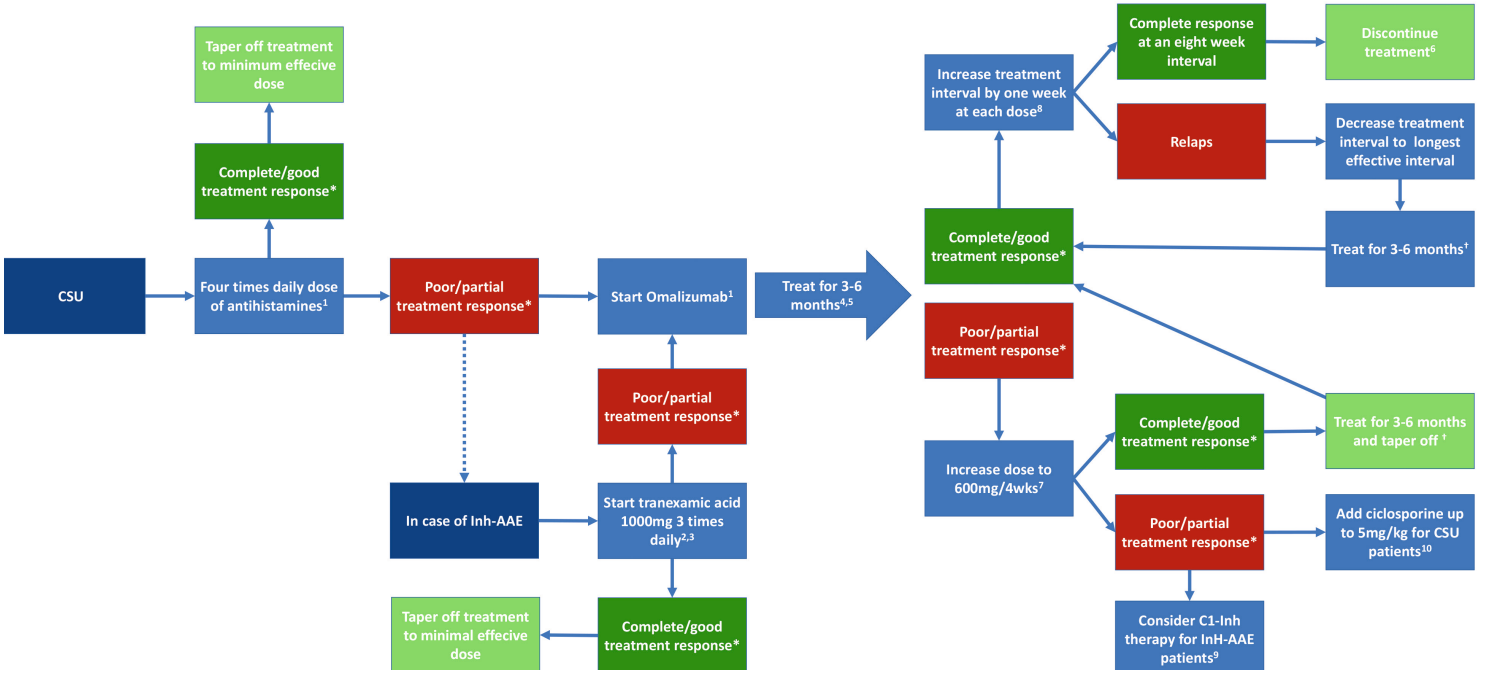


Figure 1. Proposed step-based model for the treatment of chronic spontaneous urticaria.

Legend: CSU; Chronic Spontaneous Urticaria, InH-AAE; idiopathic nonhistaminergic acquired angioedema, C1-Inh: C1-esterase inhibitor.

*Complete response: UAS7=0, UCT=16 Good response: UAS7 ≤6 or UCT ≥12. Partial response: a decrease in the UAS7-score of 10 points or a rise in UCT score of 3, but not reaching UAS7 ≤6 or UCT ≥12. Poor response: Patients who did meet these criteria.

†Expert opinion, no published data available, more research is required.

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CHAPTER 11

Nederlandse samenvatting

Deel I. Optimalisatie van omalizumab behandeling bij volwassenen en kinderen met chronische urticaria.

Urticaria, ook wel netelroos of galbulten genoemd, is een huidziekte die gekenmerkt wordt door vluchtige, intens jeukende bulten. Zelden is er een onderliggende oorzaak aan te tonen, zoals een allergie voor voedsel of medicijnen. In sommige gevallen is er sprake van een externe fysische trigger zoals frictie , kou of inspanning, dan is er sprake van induceerbare urticaria (CindU), ook hier wordt zelden een oorzaak voor gevonden. Wanneer de ziekte langer dan zes weken duurt, wordt het chronische urticaria genoemd. Ongeveer de helft van de patiënten met urticaria heeft ook recidiverende zwellingen (angio-oedeem), wat ook onder de noemer chronische urticaria wordt geschaard. Het mechanisme achter urticaria en angio-oedeem is niet geheel duidelijk, maar activatie van de mestcel met vrijkomen van histamine wordt als een belangrijk mechanisme gezien. Angio-oedeem zonder urticaria kan naast histamine ook door een teveel aan bradykinine worden veroorzaakt. In de behandeling van urticaria en angio-oedeem is de eerste stap: eenmaal daags antihistamine. Bij onvoldoende effect wordt de dosering verhoogd naar viermaal daags. Omalizumab is sinds 2014 goedgekeurd als toegevoegde (add-on) medicatie voor patiënten met chronische urticaria (CU) en idiopathisch angio-oedeem (AO) waarbij behandeling met een viervoudige dosis van antihistamine niet effectief is. Op basis van de resultaten van gerandomiseerde gecontroleerde studies bevelen nationale en internationale richtlijnen een standaarddosis van 300 mg omalizumab om de vier weken aan.

Ongeveer één op de vier patiënten reageert echter niet of onvoldoende op de standaarddosis omalizumab. Verhoging van de dosering omalizumab kan in deze gevallen uitkomst bieden, maar er is weinig bekend over de effectiviteit en veiligheid van dosis verhoging. Tevens is er momenteel geen consensus over of en hoe de behandeling met omalizumab afgebouwd kan worden bij patiënten met een goed therapie effect. Behandeldosis, behandelingsinterval, behandelingsduur en behandelbeëindiging verschillen tussen landen en worden niet behandeld in internationale richtlijnen door een gebrek aan klinische studies. In het eerste deel van deze samenvatting bespreken we de resultaten van het verbeteren van therapie, met als doel het optimaliseren van het behandelstrategie, waarbij de verschillende stappen in de behandeling elkaar opvolgen.

In **hoofdstuk 2** presenteren we de langetermijnprestaties van omalizumab in de dagelijkse praktijk geanalyseerd in een multicenter volwassen CU-cohort met behulp van drug survival analyse; de duur dat patiënten een bepaald geneesmiddel gebruiken. Het bleek dat de algehele drug survival van omalizumab (het percentage patiënten dat omalizumab gebruikte) daalde van 76% naar 37% in de eerste 5 jaar en voornamelijk bepaald werd door goed gecontroleerde ziekteactiviteit. Dit geeft aan dat bij de meerderheid van de patiënten de behandeling met omalizumab wordt beëindigt vanwege ziekteremissie en niet vanwege bijwerkingen of ineffectiviteit. Dit laat ook zien dat meer dan een derde van de patiënten een behandeling van langer dan 5 jaar

nodig heeft. Patiënten met een snel behandeleffect na het starten van de omalizumab behandeling, hadden een hogere kans om de behandeling te stoppen vanwege goed gecontroleerde ziekte. Patiënten met gelijktijdig een auto-immuunziekte of een langere ziekte duur (van twee jaar of langer), hadden daarentegen een lagere kans hadden om de behandeling te stoppen vanwege goed gecontroleerde ziekte.

De langetermijnprestaties van omalizumab bij kinderen in een multicenter pediatrische CU-populatie waren het onderwerp in **hoofdstuk 3** en lieten een vergelijkbare drug survival zien als bij volwassen patiënten. De 1- en 2-jaar drug survival rate waren respectievelijk 62% en 50%. Goed gecontroleerde ziekteactiviteit was ook bij kinderen de meest voorkomende reden voor het stoppen van de behandeling en wijst op een vergelijkbaar gunstig effect van de behandeling. In **hoofdstuk 4** laten we in een studie in de dagelijkse praktijk zien dat 27% van de patiënten met hogere dosis omalizumab behandeld wordt i.v.m. onvoldoende effect op de standaard dosering van omalizumab (300 mg per 4 weken). Hogere doses van omalizumab (tot 450 mg/600 mg per 2 weken) resulteerden tot goed of zeer goed effect bij 61% van deze patiënten zonder een toename van bijwerkingen in ernst of aantal.

In **hoofdstuk 5** laten we zien dat bij 82% van alle patiënten met een goed of volledig effect het behandelinterval van omalizumab kon worden verlengd tot voorbij het standaard interval van 4 weken. De goede ziektecontrole bleef hierbij behouden. Er werd onderscheid gemaakt tussen patiënten waarbij de ziekte in complete remissie was waardoor er geen behandeling meer noodzakelijk was en patiënten met een nog actieve ziekte, wat geconcludeerd werd door weer opkomende urticaria klachten na stoppen van de behandeling of bij een te lang behandelinterval. Het bleek dat het gemiddelde behandel interval van patiënten met een actieve ziekte 7 weken was i.p.v. de voorgeschreven 4 weken, wat leidt tot een vermindering in zowel de medicijnkosten als ziekenhuisbezoeken. Patiënten met een snel effect van de behandeling (< 4 weken) hadden een hogere kans op een succesvolle verlenging van de behandelintervallen. Ook konden patiënten die initieel met de omalizumab behandeling staakt waren, maar vanwege terugkerende ziekte de behandeling moesten herstarten, in een tweede behandelingsperiode vaker een behandelinterval van langer dan 4 weken behalen.

In **hoofdstuk 6** hebben we de klinische kenmerken van patiënten met voornamelijk angio-oedeem in het kader van chronische spontane urticaria (CSU) gekarakteriseerd. We richtten ons op idiopathisch AE, met en zonder urticaria, en onderzochten de ziekte-ernst door middel van analyse van aanvalsfrequentie en ziektecontrole gerelateerd aan het effect van onderhoudsbehandeling. In een deel van onze populatie (27%) werd geen profylactische behandeling gebruikt, waarvan de meerderheid aanvalsvrij bleef gedurende de follow-up periode, wat suggereert dat de ernst en het natuurlijke beloop van de ziekte relatief mild kan zijn. Dit werd ook ondersteund door onze bevinding dat 81% van deze groep geen aanvalsbehandeling gebruikte.

Profylactische antihistamine monotherapie, de eerste stap in behandeling van AO, werd gebruikt bij 59% van de patiënten en resulteerde in een goede ziektecontrole bij 77%. Add-on behandeling, meestal met omalizumab, werd voorgeschreven bij 14% van de patiënten. Moeilijk te behandelen ziekte werd waargenomen bij 8% van de patiënten, met geen behandel effect van viermaal de standaard dosering antihistaminica en/of behandeling met omalizumab. Deze bevindingen waren vergelijkbaar voor patiënten met idiopathisch AE met of zonder urticaria.

Deel II. Verkenning van het pathomechanisme van CU en het werkingsmechanisme van omalizumab en recombinant humane C1-esteraseremmer (rhC1-INH).

Tot op heden is de exacte pathogenese van CU en AO en het werkingsmechanisme van omalizumab bij chronische urticaria niet volledige volledig bekend. In een prospectieve studie, uitgevoerd met 30 patiënten met ernstige CSU werd de klinische effectiviteit van omalizumab in relatie tot immunologische en cutane veranderingen onderzocht. De resultaten werden gerapporteerd in **hoofdstuk 7 en 8**. Vijftien patiënten (50%) vertoonden een goed of zelfs volledig behandel effect na zes maanden behandeling. Bij 20% (n = 6) was dit klinische effect al zichtbaar binnen de eerste maand van behandeling, wat wijst op een snel effect van omalizumab bij een deel van de patiënten.

In **hoofdstuk 7** werd de mogelijke betrokkenheid van het complement systeem bij de pathogenese van CU onderzocht in serum en huidbiopten van patiënten voor de start en tijdens de behandeling. Huidbiopten van de aangedane huid voor behandeling vertoonden complementafzetting (C4d) in de papillaire dermis van bloedvaten in 53% (16/30) van de patiënten vergeleken met onaangedane huid. Dit wijst erop dat lokale complementactivatie mogelijk een rol speelt in het pathomechanisme van CSU. Bovendien waren op baseline C5a-niveaus in het perifere bloed bij CSU patiënten verhoogd in vergelijking met gezonde controles, wat ook wijst op complementactivatie bij actieve CSU.

Mestcellen worden beschouwd als de belangrijkste effectorcellen bij CSU hoewel andere leukocyten (met name die met FcεRI-receptoren) ook hierbij betrokken kunnen zijn. In **hoofdstuk 8** beschreven we het beloop van FcεRI-expressie van basofielen, eosinofielen, monocyten en drie soorten dendritische cellen voor en tijdens de behandeling met omalizumab van patiënten met CSU. Opmerkelijk was dat de FcεRI-expressie op basofielen al binnen zeven dagen na toediening van omalizumab significant afnam. Onverwacht was de bevinding dat de anti-IgE-geïnduceerde degranulatie significant toenam. Dit zou kunnen komen doordat een actieve ziekte de basofielen uitput en ze daarom minder gevoelig zijn voor anti-IgE-geïnduceerde degranulatie. Deze effecten bleven ten minste 3 maanden na stopzetting van de behandeling bestaan, wat mogelijk het lang aanhoudende effect van omalizumab kan verklaren. Echter geen van deze bevindingen kon het klinische effect van omalizumab behandeling voorspellen, aangezien er geen significante verschillen werden gevonden tussen patiënten met en zonder behandeleffect. De afgenomen FcεRI-expressie na omalizumab werd ook

waargenomen bij de sommige soorten dendritische cellen, maar niet bij monocyten. Deze bevindingen konden echter evenmin het therapie effect voorspellen. Verder onderzoek is nodig naar de mogelijke klinische relevantie van deze bevindingen.

Bij de pathogenese van AO worden twee verschillende belangrijke pathomechanismen vermoed die betrokken kunnen zijn bij het ontstaan van de typische acute zwellingen, namelijk via de histamine-route door o.a. mestcel granulatie en de bradykinine-route via o.a. C1-esteraseremmer- deficiëntie. Vaak is bradykinine gemedieerd angio-oedeem een erfelijke vorm van angio-oedeem, waarbij een defect aan C1-esteraseremmer ervoor zorgt dat bradykinine niet wordt afgebroken en zich opstapelt. Bradykinine gemedieerd AO kan echter ook niet-aangeboren zijn of door ACE-remmer gebruik worden veroorzaakt. AO bij patiënten zonder bewezen C1-esteraseremmer-deficiëntie en waarvoor geen onderliggende oorzaak kan worden gevonden, wordt beschouwd als idiopathisch AO. Bij patiënten met idiopathisch AO die niet reageren op antihistamine therapie (idiopathische niet-histaminerge AE (InH-AAE)), wordt add-on behandeling met omalizumab gestart zoals vermeld in de CU-richtlijnen. In onze studie beschreven in **hoofdstuk 6** reageert 44% van de patiënten met idiopathisch AO niet op omalizumab, wat meer is dan bij CU patiënten met galbulten als hoofdsymptoom (27%). Onze hypothese was dat het toedienen van C1-esteraseremmer, dat een zeer effectieve behandeling is bij erfelijk angio-oedeem, ook effectief zou kunnen zijn bij patiënten met idiopathisch angio-oedeem die niet reageren op antihistaminica al of niet in combinatie met omalizumab. Daarom hebben we prospectief het effect van profylactische therapie met rhC1-INH en het mechanisme erachter onderzocht bij patiënten met InH-AAE, zoals gepresenteerd in **hoofdstuk 9**. Eén van de zes patiënten vertoonde een vermindering van 85% in de frequentie van aanvallen. Deze patiënt had ook een goed therapie effect van C1-INH gemaakt uit plasma, maar niet op omalizumab. Helaas konden geen van de gemeten biomarkers bij aanvang en tijdens de behandeling worden gerelateerd aan het behandeleffect en kan niet worden voorspeld welke patiënt hier gunstig op zal gaan reageren. Van de vier patiënten die niet reageerden op rhC1-INH en na de studie werden behandeld met omalizumab, vertoonden twee een goede klinische effectiviteit, wat wijst op verschillende pathomechanismen bij AE-patiënten.

CHAPTER 12

List of abbreviations

Contributing authors

Acknowledgments

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LIST OF ABBREVIATIONS

AAE	Acquired AE
AAE-C1-INH	Acquired C1 inhibitor deficiency
AAS	Angioedema Activity Score
ACEI-AE	Angiotensin-converting enzyme inhibitors induced angioedema
AE	Angioedema.
CindU	Chronic inducible urticaria;
CSU	Chronic spontaneous urticaria;
CSU-AE	CSU with mainly angioedema
CSU-wheals	CSU with mainly symptoms of wheals
CT	Continuous treatment;
CU	Chronic urticaria
EDTA	Ethylenediaminetetraacetic acid
EMC	Erasmus Medical Center
GR-RS1	Good response restart (first treatment episode)
GR-RS2	Good response restart (second treatment episode)
GR-stop	Good response stop (no restart)
HAE	Hereditary angioedema
IH-AAE	Idiopathic histaminergic AAE
Inh-AAE	Idiopathic Non-histaminergic Aquired Angioedema
IQR	Interquartile range;
mDC	Myeloid dendritic cells
MID	Minimal important difference
n.a.	Not applicable
pDC	Plasmacytoid dendritic cells
PR	Treatment stopped due to poor response
RCT	Randomized Controlled Trial
SD	Standard deviation
UAS7	Urticaria Activity Score summed over seven days
UCT	Urticaria Control Test
UMCU	University Medical Centre Utrecht
WKZ	Wilhelmina Children's Hospital

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