CLINICAL CHARACTERISTICS AND TREATMENT OF CHRONIC URTICARIA AND ANGIOEDEMA:

DWELLING ON SWELLING, DEALING WITH WHEALING

MIGNON VAN DEN ELZEN

"An expert is a person who has made all the mistakes
which can be made in a very narrow field."
(Niels Bohr, Nobel Prize winner Physics 1922)

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CLINICAL CHARACTERISTICS AND TREATMENT OF CHRONIC URTICARIA AND ANGIOEDEMA:

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KLINISCHE KENMERKEN EN BEHANDELING VAN CHRONISCHE URTICARIA EN ANGIO-OEDEEM
(met een samenvatting in het Nederlands)

Proefschrift

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CHAPTER 1:

GENERAL INTRODUCTION

OBJECTIVES OF THIS THESIS

The general objective of this thesis is to gain insight in treatment of adult patients with urticaria with or without angioedema, and patients with angioedema with or without urticaria.

The specific objectives of this thesis are 1) to gain more insight in the clinical characteristics of patients with angioedema with or without urticaria, 2) to investigate the effectiveness and safety of treatment retrospectively, quantify the need for additional treatment options, and provide an overview of available therapeutic options, 3) to evaluate the effectiveness and safety of additional treatment with omalizumab prospectively, and 4) to gain more insight in the mechanism of action of omalizumab being a new treatment option for urticaria.

Angioedema and urticaria

Heinrich Irenäus Quincke was born on August 26, 1842, in Frankfurt/Oder. He moved with his parents to Berlin, where he was schooled as carpenter, and acquired his medical degree. Later he became professor in internal medicine. His assistant Eugen Dinckelacker wrote a doctorate's thesis about acute, circumscribed edema of skin and mucosa, and in 1882 Quincke published an article about a condition he regarded as 'angioneurotic edema'.[1–3] He was the first to give a clear description of the symptoms we now know as angioedema. Ever since, his name is tightly linked with angioedema.[2,3]

Quincke described swellings of limbs, body, or face, with a diameter of 2-10 cm. Mucosa of the palate, pharynx, and larynx could also be affected, in some cases causing problems with breathing. The affected skin looks a little pale, and no itch or fever occur. Swellings occur of a sudden, often at more than one location simultaneously, and disappear in hours to days. Physical exercise and cooling are triggers. The swellings can relapse, and they can be familial.[2,3] Quincke thought it to be a "vasomotor neurosis" closely related to another "neurotic" disease, urticaria. This was questioned by William Osler in 1888, when he described a condition characterized by 3 features: local swellings in various parts of the body, invariable gastrointestinal disturbances, and a strongly marked hereditary disposition for the disease.[4,5] This was later named hereditary angioedema (HAE).[4,6]

In 2014, 132 years after Quinckes first description of angioedema, angioedema was defined as localized and self-limiting edema of the subcutaneous and submucosal tissue, due to a temporary increase in vascular permeability caused by the release of vasoactive mediator(s).[6] It frequently occurs as part of urticaria, which is characterized by the sudden appearance of wheals, angioedema, or both.[6,7] A wheal consists of three typical features: 1) it is characterized by a central swelling of variable size, almost invariably surrounded by a reflex erythema, 2) it is associated with itching or sometimes a burning sensation, and 3) it has a fleeting nature, with the skin returning to its normal appearance, usually within 1–24 hours. Sometimes wheals resolve even more quickly.[7] In the latest European guideline chronic spontaneous urticaria (CSU), formerly known as – and in the US guideline still named – chronic idiopathic urticaria (CIU), is defined as the appearance of wheals, angioedema, or both for a period of at least 6 weeks, due to known or unknown causes.[7] This indicates that angioedema without urticaria should also be named CSU or CIU. In this thesis we distinguish CSU from angioedema without urticaria, since the latter also includes other types of angioedema with a different expected pathophysiological background (see below).

How CSU and angioedema without urticaria affect the population

The lifetime prevalence of all types of urticaria is estimated between 8.8% and 22.3%.[8] For CSU a period prevalence of 0.8% was found in 1 year in Germany.[9] The annual prevalence of CSU was estimated at 0.38% in Italy in 2013, and seems to be increasing.[10] It has been

estimated that 29–65 % of patients with CSU have urticaria only, 33–67 % have both urticaria and angioedema, and 1–13 % have angioedema only.[8,11] It is thought that 66–93% of chronic urticaria patients have CSU, the others have any form of inducible urticaria. The majority of studies show that women suffer from CSU nearly twice as often as men do. The disease can occur in all age groups and has a peak incidence between 20 and 40 years in most studies. This implies that many patients are affected during important years of their working age. No apparent relationship has been found between the prevalence of CSU and level of education, income, occupation, place of residence or ethnic background.[8]

The natural course of CSU is self-limiting, but the time to remission varies widely.[8] In 50% of the general CSU patients symptoms will resolve within 6 months after its onset, in an additional 20% it will resolve within another year (36 months disease duration in total), in 20% within 5 years and in the remaining 10% it will last longer than 5 years with exceptional cases of up to 50 years.[8,12 8,12] In tertiary referral centers such as the UMC Utrecht it is possible that patients with more severe or prolonged disease are referred. Two Dutch studies support this: In a study in 78 CSU patients in the university hospital in Amsterdam, 47% were free of symptoms after 1 year.[13] However, in a different study in 153 CSU patients in the university hospital in Nijmegen, only 34% achieved remission 5 years after the time of diagnosis, and 49% after 10 years. The duration of symptoms prior to diagnosis was an additional 4.4 years.[14] The time to remission is shown to be longer in patients with moderate to severe disease, in patients with angioedema, in those with a positive response to their own serum as tested by the autologous serum skin test, or in those with anti-thyroid antibodies, compared to patients without these characteristics.[8,15]

Quality of life is impaired in CSU patients. Pruritus and pain, in addition to the unpredictability of attacks, a lack of sleep, fatigue due to the disease and/or as side effect of treatment, and disfigurement, lead to a feeling of loss of control over their lives.[8] In comparison with other skin diseases, CSU was repeatedly shown to cause a strong reduction in quality of life,[8,16] especially in terms of discomfort and depression or anxiety.[17] Several tools are available to measure quality of life, including dermatology-specific and disease-specific questionnaires.[18–21]the Dermatology Life Quality Index (DLQI The International and Dutch guidelines for CSU recommend to measure quality of life in addition to disease activity in all CSU patients – including patients with angioedema without wheals – in order to gain a careful estimate of the impact of CSU on patients' lives.[7,22,23]

Suggested mechanisms of CSU and angioedema without urticaria

Although CSU is a relatively common disease with a high impact on patients' lives, it is surprising how little we know about pathophysiology and etiology. [24] In both CSU and other forms of urticaria it was shown that when blisters were induced at the site of urticarial lesions, the derived skin tissue fluid showed elevated histamine levels. Since patients with

CSU have normal plasma histamine levels during active disease, this indicates that histamine is produced locally at the site of the skin lesions.[25] Activation of dermal mast cells and the release of their inflammatory mediators, including but not limited to histamine, are regarded as the final common pathway. This is supported by clinical responsiveness of urticaria to H1-antihistamines[26] and to omalizumab (see below). Mast cell degranulation can result from cross-linkage of immunoglobulin (Ig)E bound to the high-affinity IgE receptors (FcεRI) on the surface of mast cells.[27] However, mast-cell activating signals in urticaria are ill-defined.[7,27] Hence, it remains unclear why mast cells degranulate, and why this only happens in the skin. A current hypothesis is that urticaria may be the result of two different pathophysiological mechanisms. One mechanism may be caused by local autoantibodies in the skin. After intradermal injection of patients' own serum during the so-called autologous serum skin test (ASST), up to 45% of CSU patients show a wheal and flare reaction, due to the presence of IgG autoantibodies.[26,28,29] These IgG autoantibodies could bind to and cross-link FcεRI on mast cells and basophils, and enable them to be triggered for degranulation.[26,27] However, it is not proven that IgG autoreactivity is a trigger for urticaria as titers of autoantibodies remain stable as patients enter natural remission.[29–31] In the other hypothesized mechanism of CSU, patients produce IgE antibodies against autoantigens, for example IgE anti-thyroperoxidase (TPO). These IgE-anti-TPO autoantibodies, when bound and activated on the surface of mast cells, might cause 'autoallergic' mast cell degranulation.[32].

In patients with urticaria and/or angioedema, a number of diseases need to be excluded from differential diagnosis.[7] One of these diseases is hereditary angioedema (HAE). HAE is an example of angioedema without urticaria, and has a different pathophysiology and different therapeutic approach as CSU has. Hereditary angioedema with C1-INH deficiency (C1-INH-HAE) is a rare disease with an estimated prevalence of 1/10 000–1/100 000 inhabitants.[6] The unpredictable, potentially fatal as well as disfiguring nature of the disease impacts the quality of life of those affected similar patients with severe asthma or Crohn's disease.[33] In HAE, angioedema occurs as a result of what is named contact system activation, which results in bradykinin-mediated angioedema as follows: Factor XII (FXII) is capable of autoactivating once it is bound to initiating surfaces. Once FXII is activated to FXIIa, it converts the proenzyme prekallikrein to the active enzyme kallikrein. There is a positive feedback in which kallikrein rapidly activates more FXII to FXIIa. Furthermore, kallikrein cleaves high molecular weight kininogen to liberate bradykinin,[34] a 9-amino acid peptide which is identified as the key mediator of angioedema.[6,34] Both FXIIa and kallikrein are inhibited by C1 inhibitor (C1INH).[34] In HAE, a C1 inhibitor (C1INH) deficiency results in instability of the contact system with facilitated release of bradykinin.[6,34] In recent years an additional cause of bradykinin-mediated HAE was described, with normal C1INH levels.[35] These patients have mutations in the gene for FXII (FXII-HAE).[6] A third example of bradykinin-mediated angioedema occurs in patients using ACE-inhibitors. Since ACE is involved in the breakdown of bradykinin, the inhibition of ACE gradually results in elevated plasma levels of bradykinin, leading to angioedema in some

patients.[6,34] In contrast to patients with C1INH deficiency, urticaria are occasionally seen accompanying the angioedema, although the angioedema predominates.[34] Angioedema caused by ACE-inhibitors is an example of acquired angioedema with a known cause. Two types of acquired angioedema with unknown cause have been described: one with response to treatment with H1-antihistamines: idiopathic histaminergic acquired angioedema, and one without: idiopathic non-histaminergic acquired angioedema. The involvement of bradykinin in ACE-inhibitor induced angioedema was confirmed in 2016, and this study also demonstrated bradykinin formation during attacks of idiopathic non-histaminergic angioedema.[36]

Clinical characteristics for HAE are well described in previous literature.[37,38] For non-HAE however, symptoms and clinical impact are not well described. Therefore, clinical characteristics, and similarities amongst subtypes of angioedema with or without urticaria are presented in **chapter 2**.

Treatment options for CSU and angioedema without urticaria

Pathophysiology suggests that bradykinin-targeted drugs, licensed to treat HAE due to C1 inhibitor deficiency, could be effective to reverse symptoms in angioedema caused by ACE-inhibitors.[6] Since involvement of bradykinin was also shown in idiopathic angioedema without urticaria, such drugs may also be effective in idiopathic angioedema. An overview of available options for several subtypes of angioedema with or without urticaria, including but not limited to angioedema caused by ACE-inhibitors, is presented in **chapter 3**.

One of the available therapeutic options for angioedema, licensed for acute attacks of HAE, is conestat alfa. This is a recombinant human C1INH (rhC1INH) is purified from milk of New Zealand White rabbits transgenic for the human C1INH gene. In two randomized, placebo-controlled studies, rhC1INH was efficacious and well tolerated in patients experiencing HAE attacks.[39] It is contraindicated in patients with known or suspected allergy to rabbits, and allergic cross-reactivity with cow's milk is theoretically possible.[40] The allergenicity of rhC1INH in patients without angioedema but with an allergy to cow's milk and/or rabbit, is addressed in **chapter 4**.

To suppress elevated levels of histamine, the international and Dutch guidelines recommend the use of second generation antihistamines as first line treatment of CSU. Second line treatment indicates up-dosing these up to fourfold.[7,22,23] Up-dosing is recommended for all types of second generation antihistamines, although this was not studied up to fourfold for all types.[22] A retrospective study of antihistamine treatment in CSU patients is shown in **chapter 5**. We evaluated treatment with antihistamines in dosages up to, or higher than fourfold. Furthermore, it is briefly addressed what proportion of the CSU population in our tertiary center is in need of third line treatment options.

In recent years a new therapeutic option for CSU has become available in the Netherlands: omalizumab. Omalizumab is a humanized monoclonal antibody which binds to free IgE, leading to a down-regulation of the FCεRI on mast cells and basophils. Consequently, these cells will no longer be able to be activated.[26] However, it has not been elucidated how this affects symptoms in CSU, hence the exact mechanism of action of omalizumab remains unclear. After a pilot study in 2008,[41] two phase II[42,43] and three phase III multicentre, randomized, placebo-controlled clinical trials[44–46] have shown that omalizumab (150 or 300 mg every 4 weeks) is efficacious and safe in treatment of CSU. This effect was seen not only in patients with IgE-anti-TPO antibodies, but could occur in all CSU patients. Omalizumab is now recommended as third-line treatment in chronic spontaneous urticaria (CSU) patients aged 12 years or older, who fail to find relief with antihistamines up to fourfold.[7,23] A systematic review and GRADE assessment of the efficacy and safety of omalizumab in CSU is presented in **chapter 6**.

As mentioned, the mechanism of action of omalizumab is not fully clear. Omalizumab reduces free IgE within 1 hour,[47] and FCεRI downregulation on basophils was noted as early as 3 days following the first dose of omalizumab in patients with allergic disease.[48] Clinical effects of omalizumab can already be demonstrated within one week and are almost optimal after 2 weeks of treatment.[44–46] Mast cells have always been considered to be the most important effector cell in CSU. However, focus is now shifting towards analysis of different FCεRI-bearing cells, including basophils. Furthermore, sera from patients with urticaria can induce degranulation of mast cells and basophils, and during this process the presence of intact complement is essential.[49] Cutaneous mast cells express the complement C5a receptor whereas pulmonary mast cells do not. This may explain why anti-IgE receptor autoantibodies in patients with urticaria, in combination with complement, would cause clinical symptoms which are limited to the skin in patients with urticaria.[50] The efficacy and mechanism of action of omalizumab, including the role of complement and basophils, was investigated and preliminary results are presented in **chapter 7**.

Although results of omalizumab in clinical trials were promising,[41–46] response to treatment in daily practice may be different. Response to treatment in clinical trials investigate efficacy, which is tested under ideal and controlled circumstances. In daily practice on the other hand, effectiveness is assessed, under usual circumstances of healthcare practice. [51] The effectiveness of omalizumab in daily practice is studied in **chapter 8.** The effect of treatment can be monitored with help of patient-reported dermatology-specific and disease-specific questionnaires with regard to disease activity,[52] disease control,[53] and quality of life.[18–21,54]

The main findings of this thesis are discussed in **chapter 9**, and summarized in **chapter 10**.

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PART I:

ANGIOEDEMA WITH OR WITHOUT URTICARIA, AND ITS TREATMENT

CHAPTER 2:

CLINICAL SIMILARITIES AMONG BRADYKININ-MEDIATED AND MAST CELL-MEDIATED SUBTYPES OF NON-HEREDITARY ANGIOEDEMA: A RETROSPECTIVE STUDY

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ABSTRACT

Background

Non-hereditary angioedema (non-HAE) is characterized by local swelling due to self-limiting, subcutaneous or submucosal extravasation of fluid, and can be divided into three subtypes. These subtypes are believed to have different pathophysiological backgrounds and are referred to in recent guidelines as bradykinin-mediated (e.g. caused by angiotensin-converting-enzyme-inhibitors), mast cell-mediated (e.g. angioedema with wheals) or idiopathic (cause unknown). Bradykinin-mediated subtypes are more closely related to hereditary angioedema than the other forms. Because clinical features of these non-HAE subtypes have not been studied in detail, we have looked at theclinical characteristics of symptoms and potential differences in clinical presentation of bradykinin-mediated and mast cell-mediated angioedema (AE) subtypes.

Methods

A questionnaire was sent to patients presenting with AE at our tertiary outpatient clinic to document clinical characteristics, potential triggers and location of AE. The severity of AE attacks was analysed using visual analogue scales (VAS).

Results

The questionnaire was returned by 106 patients, of which 104 were included in the analysis. AE with wheals, idiopathic AE, and drug-associated AE occurred in 64 (62%), 25 (24%) and 15 patients (14%) respectively. Most patients (62%) reported prodromal symptoms while 63% reported multiple locations for an attack. Face and oropharynx were the main locations of AE attacks of any subtype while swelling was the symptom most frequently reported as severe. Overall severity of the last attack was indicated as severe by 68% of the patients. There were no differences between the subgroups.

Conclusion

This similarity in clinical presentation raises the possibility that ACEi-induced, mast cell-mediated and idiopathic AE share common pathways.

BACKGROUND

Angioedema (AE) is caused by a rapid local increase in permeability of capillaries and venules with subsequent extravasation of fluid into the interstitial space, which becomes clinically manifest as self-limiting, localized subcutaneous or submucosal swellings. AE is classified into several subtypes.[1,2,3] The first step in the classification is to differentiate AE with wheals from AE without wheals. AE with wheals can be diagnosed as chronic spontaneous urticaria (CSU) or chronic inducible urticaria (CINDU), and is presumably mast-cell mediated[2], although treatment with (high doses of) antihistamines sometimes does not always lead to complete symptom relief.[4] AE may occur in all forms of CSU and CINDU, except dermographism.[3] It can be caused or aggravated by medical drugs such as NSAIDs and antibiotics.[2,5] AE without wheals can be classified further into hereditary and acquired types. Both can be caused by a C1-inhibitor deficiency, in which case a diagnosis of hereditary AE (C1-INH-HAE) or acquired AE (C1-INH-AAE) can be made. HAE can subsequently be divided in to three types, C1-INH-HAE types I and II caused by C1 inhibitor deficiency and hereditary AE with factor XII mutations or of unknown origin (formerly known as type III HAE), which causes enhanced generation of bradykinin.[1,6]

AE without wheals can also be associated with the use of angiotensin-converting-enzyme-inhibitors (ACEi). ACEi causes AE which is presumably bradykinin-mediated and is more closely related to hereditary angioedema (HAE) than the other forms.[6] Finally, idiopathic AE is diagnosed when all other causes have been excluded.[2,3,4,6] Idiopathic AE can be either histaminergic or non-histaminergic, based on the response to antihistamines.[6] It is unclear to what extent idiopathic AE has a similar pathogenesis to angioedema in patients suffering with chronic spontaneous urticaria (CSU).

Clinical characteristics for HAE are well-described in previous literature.[1,7] For non-HAE however, symptoms and clinical impact are not well described. In this study, a large unselected group of non-HAE patients was categorized into the three AE subtypes: AE with wheals (mast-cell mediated), ACEi-induced AE (bradykinin-mediated) and idiopathic AE (unknown cause). The clinical characteristics, locations and impact of the disease for each subtype were documented. In addition, we adapted the VAS tools developed for HAE and supplemented them with extra symptom scores, and used these to assess severity and type of symptoms of the last AE attack in these patients.[8,9]

METHODS

Patients

All patients visiting the outpatient clinic of the Department of Dermatology and Allergology of the UMC Utrecht between October 2007 and December 2010 for evaluation of angioedema were selected. The diagnosis AE was based on a history of bouts of mucocutaneous or subcutaneous swellings. All case records were checked by one of the investigators to verify

the diagnosis. Exclusion criteria were (a) decreased C4-value or proven HAE or AAE due to C1-inhibitor deficiency; (b) patients known to have comorbid malignancy requiring active treatment, because we wanted to avoid any unnecessary discomfort for patients with this disease; and (c) incapability of a patient to fill out the questionnaire. Four patients with AE were excluded from the study because they met one of these criteria, 2 with malignancy, 1 with a cerebrovascular accident and 1 with psychiatric disease.
This study was approved by the ethics committee of the UMC Utrecht, protocol number 13-241/C.

Questionnaires

Questionnaires were sent to all selected patients to evaluate the subtype and characteristics of their AE. A written reminder was sent topatients who failed to reply after 2 weeks. Two weeks after the first reminder patients who had still failed to respond were contacted by phone and asked to complete the questionnaire. Of the 165 patients to whom the questionnaire was sent , 106 (64%) returned it. One patient did not meet inclusion criteria and the remaining 105 patients were included in the study. Reasons for not filling out the questionnaire included: lack of time (n=15), lack of a recent AE attack (n=16), as ascertained during a phone interview, or were unknown (n=28).

Evaluation of symptoms and locations

The questionnaire consisted of 17 general questions related to the frequency and impact on daily life, locations involved and treatment of AE attacks. The questionnaires were provided with images to mark the location(s) involved during the last attack. We elected to evaluate the last attack rather than, for instance, the most severe one in order to minimise recall bias. Moreover, we restricted the analysis to patients who had visited the clinic within the recruitment period. In addition, the questionnaire was designed in such a way that answers were double-checked whenever possible. For example, location of the last attack by questionnaire was verified by asking patients to indicate this location also on graphs.

Severity of symptoms

Furthermore the questionnaire contained a series of symptom-specific visual analogue scale (VAS)[6,7] to assess the severity of the last attack of AE. A value of <20mm was considered to represent a symptom or attack of minimal severity, >20mm-50mm a moderate symptom or attack, and >50mm a severe symptom or attack. Different sets of VAS[7] were used and expanded for different anatomical locations of an AE attack; namely the face (eyelids, cheeks, lips and ears), the oropharyngeal cavity (tongue, throat, uvula and vocal cords), the extremities (arms, hands, legs, feet and trunk - also referred to as peripheral locations) and the abdomen.

Subtyping of AE

The following, clinically defined, subgroups of AE were discriminated in patients who had completed the questionnaire:

1) AE associated with wheals, (AE with wheals): Patients were included in this category when they had associated wheals or pruritus alone or if they scored at least 20mm on a VAS for pruritus when scoring the severity of their last AE attack. In case of a relation with NSAID or ACEi use they were considered to be drug-associated (see next subgroup).

2) AE associated with the use of drugs as NSAID, antibiotics or ACEi: Patients were included in this category when AE became manifest for the first time after using these drugs. As we did not identify patients with AE induced by NSAIDs or antibiotics, this subgroup is further referred to as ACEi-induced AE.

3) AE associated with other diseases such as auto-immune disease: This category is further referred to as AE due to other causes. After subtyping, this subgroup resulted in only one patient with AE related to food exposure, who was excluded from further analysis as the group was not sufficiently large to produce statistically reliable results.

4) Idiopathic AE was diagnosed when no other cause for AE could be identified.

Data management

Differences between the different subgroups were evaluated by analyzing the answers to the general questions in the questionnaire and the VAS scores of the last AE attack. The VAS forms were used to assess the location as well as the severity and type of symptoms. Analysis of the time to 50% reduction and to minimal symptoms of the last attack was carried out using the answers to that specific question.

All patients who completed and returned the questionnaire were allocated to subgroups. After exclusion of the patient in the group of AE due to other causes, the final number of patients, 104 in total, were included in the analysis. Descriptive statistics were used to describe the data. The data was presented as median values with interquartile ranges.

RESULTS

Patient characteristics and subtypes of AE

The median age of the 104 patients included in the study was 55 years and 67 (64%) were female. The median age of onset of AE was 46 years. 12 patients had a positive family history of AE, but none had a proven C1-inhibitor deficiency. 64 patients (62%) had AE with wheals, 15 (14%) had ACEi-induced AE, and 25 (24%) had idiopathic AE. Demographic data of the different subgroups is listed in Table 1.

Table 1: Clinical characteristics of non–HAE patients (n=104)

	Total group n=104		Idiopathic n=25		AE with wheals n=64		ACEi-induced n=15	
Male (%)	37	(36%)	14	(56%)	15	(23%)	8	(53%)
Age (years)	55	(42-65)	61	(44-67)	50	(40-61)	64	(58-67)
Family history with AE	12	(12%)	1	(4%)	11	(17%)	0	(0%)
Family history unknown	15	(14%)	4	(16%)	7	(11%)	4	(27%)
Age of onset	46	(35-60)	47	(36-61)	41	(33-54)	59	(63)

Data is presented as numbers and percentages or median values with interquartile ranges

Locations of AE attacks

All reported locations are shown in Figure 1a. In all subgroups, the face was mentioned most frequently as a location of attacks (96 [92%]). The second most frequent location for all subgroups was the oropharynx (68 [65%]). 47 (45%) mentioned a previous peripheral attack. 10 (10%) mentioned at least one attack in the genital location, and 20 (19%) reported at least one attack in the abdominal location. Figure 1b shows a breakdown of the frequency of facial attacks and figure 1c shows a breakdown of the frequency of oropharyngeal locations of AE attacks. The lips are the most frequently involved location in the face, while the ears are the least involved. No patients in the idiopathic AE subgroup reported an attack of the ears. The tongue and pharynx were the most frequent locations of attacks in the oropharynx, with 49% and 35% of all patients suffering with attacks at these locations, respectively. Laryngeal attacks occurred in 13% of patients. Laryngeal locations were less frequent in the idiopathic AE subgroup (1 patient (4%) with idiopathic AE) as compared to 15% for AE with wheals and 20% with ACEi-induced AE. In ACEi-induced AE, uvular locations were less frequent (7%) compared to idiopathic AE (16%) and AE with wheals (25%). All different anatomical locations involved in at least one historical attack mentioned by the patients are shown in Additional File 1.

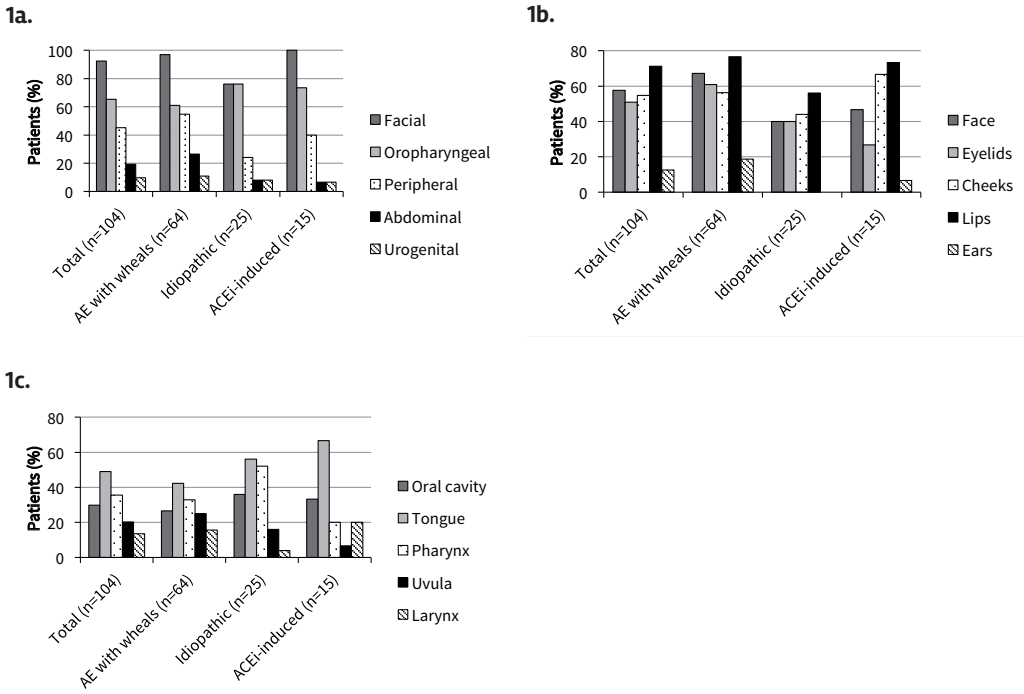
Sixty-six of the 104 patients (63%) reported that they had suffered AE attacks at multiple locations. Patients with idiopathic AE reported this less frequently (up to 40% of all attacks were at multiple locations) than the other groups. Characteristics of angioedema attacks are summarized in Table 2. 62% of the patients reported prodromal symptoms preceding an attack of AE. This included paraesthesia, pruritus or erythema. Only 40% of the patients with ACEi-induced AE reported prodromal symptoms (n=6, of which n=3 reported pruritus).

High severity and long duration of the last AE attack

VAS scores for the last AE attack were completed by 99 of the 104 patients. 67 (68 %) rated the overall severity of the last attack as ≥50 mm on VAS for at least one involved location. For facial and oropharyngeal locations, swelling was the most frequent symptom reported as ≥ 50 mm (Figure 2a and b). In addition, many patients with oropharyngeal AE reported severe

Figure 1: Locations of AE attacks

Reported locations of attacks for the total group (n=104) and by subtype of angioedema for all locations, presented as percentages (a). A breakdown of facial (b) and oropharyngeal attacks (c) is also presented.



(VAS ≥50 mm) difficulties in swallowing (55%), severe changes in speech (41%) and severely impaired breathing (37%). Pruritus and swelling were the symptoms most frequently scored severe for peripheral AE (Figure 2c). Ten patients with peripheral AE also reported severe pain , but this was not reported by idiopathic AE with peripheral locations. VAS scores did not differ between AE subgroups for oropharyngeal attacks.

Median time to 50% resolution was 23 hours for facial attacks, 15 hours for oropharyngeal attacks, 30 hours for peripheral attacks, and 9 hours for abdominal attacks (Table 2). Median time to complete resolution was 57 hours for facial lesions, 38 hours for oropharyngeal attacks, 66.6 hours for peripheral attacks, and 33 hours for abdominal attacks.

Impact of AE attacks on daily life

Of the total group, a minority showed to have a high frequency of attacks: 20 Twenty patients (19%) reported having suffered an attack more than once a week, 23 (22%) more than once a month, 32 (31%) more than once a year, and 22 patients (21%) less than once a year. The frequency of attacks was comparable in most subgroups except that, compared to other subgroups, in the idiopathic group more patients reported having suffered an attack less than once a year (17-20% versus 32%, respectively).

Medical burden and social impact on daily life of having AE are summarized in Table 3. Of all 104 patients, 22 (21%) reported having sought medical advice when suffering with an AE attack. Twenty-nine patients (28%) reported having been admitted to the hospital at least once because of an AE attack of which 5 (5%) had been admitted to an Intensive Care Unit at least once. A majority of the patients (77%) reported having used antihistamines on demand during an AE attack. Especially in AE with wheals, oral corticosteroids were used on demand as well. Sixty-one (59%) used antihistamines as prophylactic medication, 29 patients (28%) reported prophylactic use of oral corticosteroids to prevent attacks of swelling.

Table 2: Characteristics of non-HAE attacks per patient (n=104)

Prodromal symptoms	64 (62)*
Pruritus	17 (16)
Paresthesia	15 (14)
Erythema	1 (1)
Other	30 (28)
Multiple locations	66 (63)
Duration of attacks	50% improved
Face	23**
Oropharynx	15.2
Peripheral	30.3
Abdominal	8.7
	100% resolved
Face	57**
Oropharynx	37.8
Peripheral	66.6
Abdominal	33
Frequency of attacks	
< 1 per year	14 (13)*
> 1 per year but <1x per month	37 (36)
> 1 per month but <1x per week	21 (20)
> 1 per week	19 (18)
Daily	4 (4)
Unknown	9 (9)

*Data are presented as numbers and percentages;

** data presented as median hours

Three of 104 patients (3%) reported having been absent from work or school because of an AE attack more than once a week, 5% more than once a month, 19 (18%) more than once a year and 61 (59%) less than once a year. 15 could not be evaluated in this respect since they neither had a job nor went to school. Surprisingly, absenteeism was seen mostly in patients with a lower attack frequency, possibly indicating that patient with frequent symptoms accept symptoms to some extent.

Table 3: Medical burden and social impact of angioedema (n=104)

Medical burden		Total group n=104 *	AE with wheals n=64 *	Idiopathic n=25 *	ACEi-induced n=15 *
Seek medical advice	Yes	22(21)	13(20)	7(28)	2(13)
	Unknown	4(4)	2(3)	2(8)	0(0)
Angioedema-related admission	To hospital	29(28)	19(30)	5(20)	5(33)
	To ICU	5(5)	2(3)	2(8)	1(7)
Social impact					
AE-related absenteeism	<1 per year	60(58)	36(53)	16(64)	10(67)
	>1x per year but <1x per month	19(18)	12(19)	6(24)	1(7)
	>1x per month but <1x per week	5(5)	4(6)	1(4)	0(0)
	>1x per week	3(3)	3(5)	0	0
	Not applicable	15(15)	9(14)	2(8)	3(20)
	Unknown	2(2)	2(3)	0(0)	1(7)

*Data are presented as numbers and percentages

DISCUSSION

To our knowledge, this is the first study to describe in detail the characteristics of angioedema attacks in a large group of non-hereditary angioedema patients, and to study potential differences between clinical subtypes. In contrast to previous literature[10], we also included information of patients suffering with AE in the presence of wheals. It has been suggested previously that angioedema resulting from bradykinin release and resulting from mast cell mediator release might show similar signs and symptoms.[2,11] In this study we show that clinical manifestations of attacks in patients with non-HAE subtypes are indeed remarkably similar in locations, frequency and severity of attacks. Our 104 patients could be allocated to the following, clinically relevant subtypes of AE: AE with wheals (n=64), ACEi-associated (n=15), AE due to other causes (n=2, both excluded from analysis) and idiopathic AE (n=25). Of these subtypes, AE with wheals is believed to be mast cell-mediated, idiopathic AE may be either mast cell- or bradykinin-mediated and ACEi-associated AE is believed to be bradykinin-mediated. Beltrami et al reported on 111 patients with ACE-inhibitor angioedema. After discontinuation of the ACE inhibitor, 46% of patients had further recurrences of angioedema, although less-frequent. These findings suggest that ACE inhibitors may certainly exacerbate angioedema in a large subset of patients but may not be the sole cause of angioedema.[12]

All patients reporting pruritus, itching of AE lesions or presence of both AE and wheals, were allocated to the AE with wheals subgroup when they did not use ACEi or NSAIDs. In drug-associated AE, ACEi, antibiotics and NSAIDs are commonly known culprits.[13] It is arguable

that NSAID-induced AE and ACEi-induced AE should be combined in a single subtype, as the first seems to be mast cell mediated and the latter bradykinin mediated. In our study this has no effects on the data as there were no patients included with AE triggered by NSAIDs. This classification is based on etiological features. It is deliberately not based on biochemical mechanisms because of a lack of evidence on this topic. These subgroups are in line with previous literature[2,3], although different classifications are sometimes used.[14]

We did not find striking differences among the demographic parameters of AE subtypes, except for a higher median age of ACEi-induced AE. This finding most likely reflects the fact that patients taking medication, especially antihypertensive drugs, are generally older.
The majority of our patients (62%) reported prodromal symptoms whereas in previous surveys among HAE patients even higher percentages ranging from 82.5-95.7% were reported.[15] Strikingly, 50% of the reported prodromal symptoms in ACEi-induced AE consisted of pruritus, which would not be expected for a bradykinin-mediated swelling. Our study was not designed to determine the interval between the reported prodromal symptoms and the onset of angioedema. To our knowledge, no study has been performed to measure prodromal symptoms in chronic urticaria patients. Also, a majority of patients reported involvement of multiple locations during attacks (63%). Face and oropharynx were the most frequent locations. AE attacks involving the extremities had the longest resolution time (almost 67 hours). We did not find gross differences in involved locations and symptoms among the various AE subtypes. For all subtypes most AE attacks occur in the face and in the oropharynx, which is consistent with recent published literature.[16] In HAE due to C1-inhibitor deficiency, extremities and the gastrointestinal tract are preferred locations.[9,17-20] In contrast, in HAE with normal C1 inhibitor,[21] attacks also occur most frequently in face and oropharynx. One may therefore speculate that C1-inhibitor deficiency per se influences the location of AE attacks, though the molecular mechanism to explain this effect of C1-inhibitor is far from clear. Another option is that in non-HAE, abdominal attacks are not as well recognized as in HAE leading to underreporting.

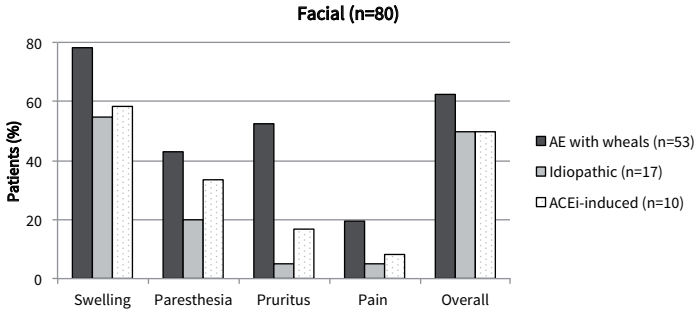
63% of the patients reported lesions on multiple locations during a single attack. Such a high frequency of multiple locations has also recently been reported in a study on peripheral attacks of HAE.[9] This might indicate that AE attacks result from a systemic trigger, rather than a local activation process. However, it cannot be excluded that local activation of biochemical processes occurs at multiple sites at the same moment. This is one of the topics in angioedema that should be explored further in future research.

Our study is retrospective in nature and therefore may have limitations due to recall bias. We tried to minimize this (as described in the methods section of this article), however the recall period of 5 years is rather long. In the vast majority of patients (83%), the last attack was reported less than two years in the past. Another limitation is the number of missing values of

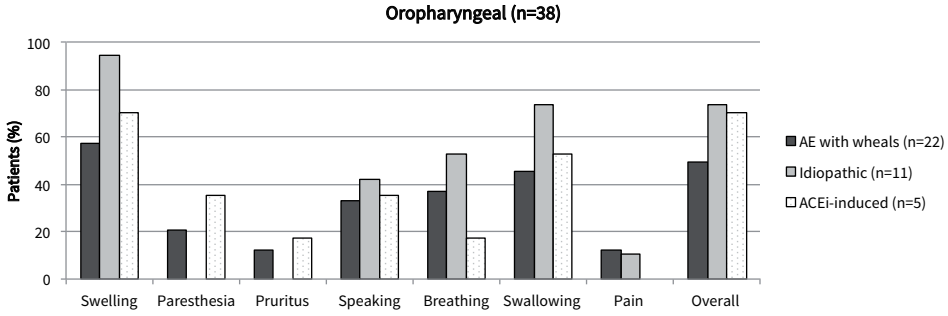
Figure 2: Symptoms per location of AE attacks

Symptoms of the last angioedema attack of each patient reported to be severe for facial (a), oropharyngeal (b) and peripheral locations (c).

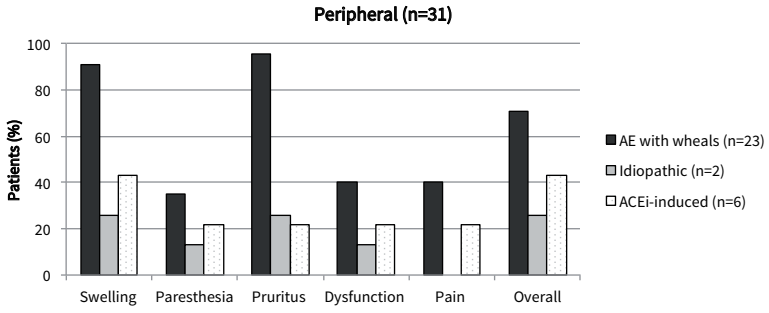
2a.



2b.



2c.



Percentages on the Y-axis represent the percentage of patients that reported the indicated symptom VAS score as ≥50 mm. Note that the number of patients varies between the different locations because the location of the last attack differs between patients.

C1-INH or C4. For idiopathic AE, blood test results were missing in 58% of patients, for AE with wheals this was the case in 48% of the patients. However, in all patients where blood tests were performed, the test results were negative. We suspect this had only a limited impact on our results.

Furthermore, patients reporting pruritis were allocated to the subgroup AE with wheals. However, pruritis was also reported as a prodromal symptom. It may be difficult to separate pruritus as a prodromal symptom from pruritus as a symptom in patients with wheals. This could lead to overestimation of the AE subtype with wheals. Additionally, the ACEi-induced AE group may be underestimated due to missing values in the medical records, causing a possible overestimation of the idiopathic AE group. We think this had limited impact on our conclusions since no major differences were observed between the subgroups.

VAS scoring provides a sensitive, reliable and validated tool for evaluation of patient reported outcome measures such as pain.[22-25] We used VAS scoring to assess severity of pain as well as that of other symptoms of the AE attacks. This analysis revealed that swelling is the dominant symptom of AE independent of subtype. Strikingly, the patients reported a high proportion (69%) of severe attacks. There is literature that suggests that VAS scores can be obtained retrospectively. In a previous study, VAS-scores were validated as an instrument for measuring HAE attack severity. VAS scores were obtained retrospectively. HAE patients reported VAS scores as if they were experiencing an acute angioedema attack at the time.[26] We feel that the use of VAS in retrospect is competent, however, as stated earlier, the recall period is rather long in our study.

Our study was not designed to evaluate the efficacy of treatment in the AE subtypes. Interestingly, 79% of the patients reported having not sought medical help in case of an acute AE attack. This is remarkable given that 65% of the patients rated their last attack as severe. Our study was not designed to evaluate the reasons why patients are reluctant to seek medical advice in case of an acute AE attack. However, one may speculate that patients with AE underestimate the severity and potential consequences of their disease. We have made similar observations in food allergic patients.[27]

We conclude that despite different etiologies, there are strong clinical similarities among different subtypes of non-HAE. Except for age, we did not find striking differences between mast cell-mediated (AE with wheals and idiopathic AE) and bradykinin-mediated AE (ACEi-induced AE), with regards to dominant symptoms, preferred locations, and prodromal symptoms. These findings support the previous suggestion that angioedema resulting from bradykinin release and resulting from mast cell mediator release show similar signs and symptoms.[2,11]

Further research should address the question whether or not these subtypes of non-HAE share a final common biochemical pathway leading to non-HAE since our questionnaires were not designed to study this. Identification of the molecular mechanisms of this pathway may provide new targets for future intervention in all non-HAE subtypes.

Acknowledgments

We thank all patients for cooperating in this study.

ADDITIONAL FILE 1: Exact numbers of all locations, by subtype

All different anatomical locations involved in at least one historical attack mentioned by the patients.

Location		Urticaria-associated (n=64)		Idiopathic (n=25)		ACEi-induced (n=15)		Total (n=104)	
		n	(%)	n	(%)	n	(%)	n	(%)
Facial	Face	43	*67*	10	*40*	7	*47*	60	*58*
	Eyelids	38	*59*	10	*40*	4	*27*	52	*50*
	Cheeks	36	*56*	11	*44*	10	*67*	57	*55*
	Lips	49	*77*	14	*56*	12	*80*	75	*72*
	Ears	12	*19*	0	*0*	1	*7*	13	*13*
Oropharyngeal	Oral cavity	17	*27*	9	*36*	5	*33*	31	*30*
	Tongue	27	*42*	14	*56*	10	*67*	51	*49*
	Pharynx	21	*33*	13	*52*	3	*20*	37	*36*
	Uvula	16	*25*	4	*16*	1	*7*	21	*20*
	Larynx	10	*16*	1	*4*	3	*20*	14	*13*
Peripheral	Arms	32	*50*	6	*24*	4	*27*	42	*40*
	Legs	28	*44*	6	*24*	6	*40*	40	*38*
Abdominal		17	*27*	2	*8*	1	*7*	20	*19*
Urogenital		7	*11*	2	*8*	1	*7*	10	*10*

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CHAPTER 3:

EFFICACY OF TREATMENT OF NON-HEREDITARY ANGIOEDEMA

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ABSTRACT

Non-hereditary angioedema (AE) with normal C1 esterase inhibitor (C1INH) can be presumably bradykinin or mast cell-mediated, or of unknown cause. In this systematic review, we searched Pubmed, Embase and Scopus to provide an overview of the efficacy of different treatment options for the abovementioned subtypes of refractory non-hereditary AE with or without wheals, and with normal C1INH. After study selection and risk of bias assessment, 61 articles were included for data extraction and analysis. Therapies were described for angiotensin converting enzyme inhibitor-induced AE (ACEi-AE), for idiopathic AE, and for AE with wheals. Described treatments consisted of ecallantide, icatibant, C1INH, fresh frozen plasma (FFP), tranexamic acid (TA), and omalizumab. Additionally, individual studies for anti-vitamin K, progestin, and methotrexate were found. Safety information was available in 26 articles. Most therapies were used off-label and in few patients. There is a need for additional studies with a high level of evidence. In conclusion, in acute attacks of ACEi-AE and idiopathic AE, treatment with icatibant, C1INH, TA, and FFP often lead to symptom relief within 2 hours with limited side effects. For prophylactic treatment of idiopathic AE and AE with wheals omalizumab, TA, and C1INH were effective and safe in the majority of patients.

INTRODUCTION

Angioedema (AE) frequently occurs as part of urticaria, a disease characterized by the development of wheals, AE, or both [1, 2]. AE with wheals, also known as chronic spontaneous urticaria (CSU), is presumably mast-cell mediated [1–3]. AE without significant wheals can be the presenting symptom of a variety of diagnoses such as hereditary AE caused by C1 esterase inhibitor (C1INH) deficiency, resulting in release of the key mediator bradykinin [2]. Accumulation of bradykinin can also be caused by the use of angiotensin-converting enzyme inhibitors (ACEi-AE) in patients with normal C1INH [2, 4]. ACEi-AE is estimated to occur in up to 0.68% of patients who receive ACE inhibitors [5]. However, a majority of patients suffer idiopathic acquired AE, which implies AE with normal C1INH with no family history of AE, in which known causes of AE have been excluded [2, 3]. It is unclear to what extent idiopathic AE is similar to angioedema with wheals (CSU) [3], or to presumably bradykinin-mediated subtypes of AE.

Second-generation antihistamines are used as prophylactic treatment of AE with wheals, and idiopathic AE [1, 2]. Antihistamines and corticosteroids, and in life-threatening cases adrenaline, represent the standard emergency room treatment of acute attacks of AE [2, 4, 6, 7]. CSU is thought to affect 0.5–1% of the global population at any given time, with an estimated 67% of patients with CSU shown to have both hives and AE, and 1–13% to have AE alone [8, 9]. In AE with wheals, daily treatment with antihistamines does not always lead to a complete absence of symptoms [1], and it is estimated that every third or fourth patient remains symptomatic even despite high-dose antihistamine treatment [8, 9]. Omalizumab is effective in patients with CSU [1, 10–15], although it has not been studied extensively in AE without wheals. Patients with ACEi-AE generally do not respond to conventional therapy [5, 6]. Pathophysiology suggests that drugs registered for HAE due to C1INH deficiency, could also be effective in ACEi-AE. Several drugs are currently available including 1) antifibrinolytic agents including tranexamic acid (TA), 2) attenuated androgens including danazol, 3) replacement of deficient proteins using fresh frozen plasma (FFP), 4) C1INH concentrates, which inhibit the formation of bradykinin, 5) the selective plasma kallikrein inhibitor ecallantide, and 6) the selective bradykinin B2 receptor antagonist icatibant [2]. Some of these drugs are licensed to treat acute attacks whereas others are used for prophylactic treatment [2]. The efficacy of these drugs in refractory AE with normal C1INH has not been fully elucidated.

This systematic literature review aims to provide an overview of therapeutical options and their efficacy in patients with AE with normal C1INH, but refractory to conventional therapy. We have distinguished between treatment of acute attacks versus prophylactic treatment, and included bradykinin-mediated and mast-cell-mediated non-hereditary AE, as well as idiopathic AE.

METHODS

This systematic literature review was conducted using the criteria mentioned in the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) statement [16].

Search strategies

Secondary evidence databases National Guideline Clearinghouse, CBO guidelines, Trip database and the Cochrane library were searched for guidelines up to April 20th, 2015, using several synonyms for the domain, angioedema, and determinant, treatment options (Table 1). Subsequently, primary evidence electronic databases Pubmed, Embase and Scopus were searched for articles up to April 20th, 2015, using the domain and determinants as previously described. Synonyms for outcome measurements were not included in the search strategy so as to maximize the yield of articles, and to allow for different outcome measures, including but not limited to time to initial or complete response, and decrease in attack frequency or severity. The search was limited by title or abstract, and in Scopus by title, abstract or keywords.

TABLE 1: Search syntax performed on April 20th 2015 in Pubmed, Embase, Scopus

Search
(Angioedema OR 'angio edema' OR angioedemas) AND (treatment OR therapy OR antihistamines OR (ciclosporine OR CsA OR cyclosporine) OR (omalizumab OR (anti IgE)) OR (danazol OR 'attenuated androgen' OR androgen) OR C1 inhibitor concentrate OR (tranexamic acid OR TTA OR cyklokapron OR AMCA OR 'trans aminomethyl cyclohexane carboxylic acid') OR biological OR antileukotrienes OR ('H2 antagonist' OR 'histamine antagonist') OR (TCA OR antidepressant) OR (icatibant OR 'bradykinin receptor antagonist') OR (MTX OR methotrexate) OR (AZA OR azathioprine OR Imuran) OR (corticosteroids OR prednisone OR glucocorticosteroids) OR Adrenaline OR sulphasalazine OR (dapson OR dapsone) OR hydroxychloroquine OR Plasmapheresis OR ('intravenous immunoglobulin' OR IVIG)) OR ('Fresh Frozen Plasma' OR FFP))

Search term 'biological' was entered as 'biologicals' in database Embase.

Inclusion and exclusion criteria

Articles were included when the described study populations suffered ACEi-AE, AE with wheals (CSU), or idiopathic AE with normal C1INH. Furthermore, only articles describing pharmacological treatment of AE were included. This included both observational studies (case report, case series) and intervention trials (cohort studies or randomized controlled trials (RCTs)). Any pharmacological treatment other than antihistamines up to fourfold, prednisolone, or adrenaline could be included. Articles were considered appropriate to be included only when sufficient details regarding type of treatment, dose, interval between doses, time to initial response, and time to maximum or complete response were described. Articles describing only ineffective therapies were described separately. For full understanding of scientific content by the authors who performed the selection of studies, only articles written in English, Dutch or German were included. Recent articles for which only title and abstract were available, such as congress abstracts, were included only if sufficient information about the patient(s), treatment regimen, and response were described. For icatibant only, when dose was missing it was assumed that 30 mg was used, due to the

packaging of this product. Therefore, these articles could be included, although the dose was shown as "not reported" in results. Articles regarding AE with wheals could only be included when treatment results specifically for AE symptoms could be extracted rather than only for the symptoms wheals and itch. Outcome measurements could differ for articles regarding an acute attack or prophylactic treatment: in acute settings, initial and complete responses refer to the resolution of a single attack of swelling, whereas in prophylactic or chronic settings this refers to a decrease in attack frequency or severity.

Studies were excluded when AE was caused by hereditary or acquired complement C1 inhibitor (C1-INH) deficiency, coagulation factor twelve (fXII) mutation formerly known as HAE type 3, or other known causes of AE, including allergy, or when AE was an adverse effect of any therapy other than ACEi.

Selection of studies

Unique titles and abstracts and subsequently full texts were screened for eligibility. Articles published in or after 2013 were screened by at least two independent reviewers (ME, MG and MB), results were compared and disagreements were discussed and resolved. Articles published before 2013 were screened by one reviewer (ME) and for assessment of unclear articles only, a second reviewer (MB) was available.

Risk of bias

Risk of bias (RoB) for each study was assessed by one reviewer (MG) and verified by a second reviewer (ME). To allow for a careful assessment of observational studies as well as intervention studies, criteria for risk of bias assessment from the Cochrane Handbook for Systematic Reviews of Interventions [17] were supplemented with items from the CARE guidelines checklist [18]. The risk of selection bias, performance bias, detection bias, attrition bias and selective reporting bias was assessed. A low risk of bias was preferred and therefore displayed as a positive finding (+), whereas a high risk of bias was undesirable and displayed as a negative finding (-). The risk of selection bias was considered low (+) for observational studies when symptoms and important clinical findings were described. The risk of performance bias was considered low (+) when the chosen treatment option and dose regimen were both recorded. The risk of detection bias was assessed with regard to (1) effect of treatment and (2) adverse events, and was considered low if this was noted in the article. In case of multiple patient groups including more than one type of AE, the risk of detection bias was considered low only when results could be extracted for the subgroups separately, and unclear (+/-) when results were described for the total group. The risk of attrition bias was low (+) when reasons for exclusion or drop-out were reported, and for controlled studies the drop outs were balanced between treatment and placebo groups. The risk for reporting bias was low (+) when all pre-specified outcomes were fully addressed in the results. Authors

of RCTs were contacted to retrieve missing trial details. All evaluations were compared and disagreements between authors were discussed and resolved.

Data extraction and synthesis

For each study, data extraction was performed by one reviewer and verified by a second reviewer (ME and MG). Data regarding study design, therapy, previous therapies, and effect of the described therapy was recorded in tables. For treatment of acute attacks and prophylactic treatment of AE, available efficacy results were described per subtype and per treatment option. Definitions for response were adopted from the original articles. Articles describing ineffective treatment options were described separately. If information about adverse effects was available, this was collected additionally for each type of treatment. A distinction between serious adverse effects (SAEs) and less severe treatment-emergent adverse events (TEAEs) was made. Additionally, only adverse events possibly, probably or definitely related to treatment were reported. Adverse effects reported by placebo treated patients were not taken into account. Due to the high amount of available case reports and low amount of controlled studies, and since outcome measures varied amongst the study studies, a meta-analysis could not be performed. Instead, results are described using narrative summary technique.

RESULTS

Search results and quality assessment

The search in secondary evidence databases yielded no available aggregated evidence. The search in Pubmed, Embase and Scopus yielded 5107 original articles (Figure 1). After screening titles and abstracts, 4952 articles were excluded. Subsequently, 155 full texts were screened for eligibility leading to exclusion of 94 further articles, including 53 articles with a lack of usable information, the use of conservative treatment in 40 articles, and overlap in study population in 1 article. The remaining 61 articles included 53 full articles and 8 (congress) abstracts. Of the 61 included articles 38 described treatment of AE in acute settings, including 3 RCTs, 2 cohort studies, 4 case series, and 29 case reports. Additionally, 26 of the 61 articles described prophylactic settings, including 1 RCT, 5 cohort studies, 9 case series, and 11 case reports. Three articles described both acute and prophylactic treatment.

All 61 articles underwent risk of bias (RoB) assessment (Tables 2 and 3). All of the 4 included RCTs had a low risk of bias. Of the 57 descriptive studies, 46 had a low risk of bias, 11 had an unclear risk of at least one type of bias. Only 26 addressed safety results with regard to efficacy outcomes.

TABLE 2: Risk of bias of acute setting studies

Acute setting				Selection bias			Performance bias		Detection bias			Attrition bias	Reporting bias	Remarks
Study	**Design**	**AE subtype**	**Usable sample size**	**Randomi-zation**	**Allocation concealment**	**Case description**	**Blinding patient and personnel**	**Inter-vention**	**Blinding outcome**	**Assessment outcome**	**Adverse events**	**Incomplete outcome data**	**Selective reporting**	
Lewis[4]	RCT	ACEi-induced	58+18	+	+	na	+	na	+	na	na	+	+	
Bas[5]	RCT	ACEi-induced	13+14	+	+	na	+	na	+	na	na	+	+	
Bernstein[7]	RCT	ACEi-induced	26+24	+	+	na	+	na	+	na	na	+	+	
Mansi[19]	Cohort	Idiopathic	26	na	na	+	na	+	na	+/-	-	+	+	*
Bouillet[20]	Cohort	Idiopathic	48	na	na	+/-	na	+/-	na	+/-	-	+	+	
Bova[21]	CS	ACEi-induced	13	na	na	+	na	+	na	+	+	na	na	
Greve[22]	CS	ACEi-induced	10	na	na	+	na	+	na	+	+	na	na	
Bas [6]	CS	ACEi-induced	8	na	na	+	na	+	na	+	+	na	na	
Hassen[23]	CS	ACEi-induced	7	na	na	+	na	+	na	+	-	na	na	
Bartal[24]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Lipski[25]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Charmillon[26]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Crooks[27]	CR	ACEi-induced	1	na	na	+	na	+	na	+	+	na	na	
Rasmussen[28]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Yates[29]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Bledsoe[30]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Volans[31]	CR	ACEi-induced	2	na	na	+	na	+	na	+	-	na	na	
Bolton[32]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Gallitelli[33]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Millot[34]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Stewart[35]	CR	ACEi-induced	2	na	na	+	na	+	na	+	-	na	na	
Bas[36]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Schmidt[37]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Dehne[38]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Nielsen[39]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	

TABLE 2 (continued)

Karim[40]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	
Acute setting				**Selection bias**			**Performance bias**		**Detection bias**			**Attrition bias**	**Reporting bias**	**Remarks**
Study	**Design**	**AE subtype**	**Usable sample size**	**Randomi-zation**	**Allocation concealment**	**Case description**	**Blinding patient and personnel**	**Inter-vention**	**Blinding outcome**	**Assessment outcome**	**Adverse events**	**Incomplete outcome data**	**Selective reporting**	
Bertazzoni[41]	CR	Idiopathic	1	na	na	+	na	+	na	+	-	na	na	
Nanda[42]	CR	Idiopathic	1	na	na	+	na	+	na	+	+	na	na	
Stahl[43]	CR	Idiopathic	1	na	na	+	na	+	na	+	-	na	na	*
Montinaro[44]	CR	Idiopathic	1	na	na	+	na	+	na	+	-	na	na	
O'Keefe[45]	CR	Idiopathic	1	na	na	+	na	+	na	+	-	na	na	
Lleonart[46]	CR	Idiopathic	1	na	na	+	na	+	na	+	+	na	na	
Sridhara[47]	CR	Idiopathic	1	na	na	+	na	+	na	+	-	na	na	
Vela Vizcaino[48]	CR	Idiopathic	1	na	na	+	na	+	na	+/-	-	na	na	*
Colas[49]	CR	Idiopathic	1	na	na	+	na	+	na	-	-	na	na	
Seoane[50]	CR	Idiopathic	1	na	na	+/-	na	+/-	na	-	-	na	na	
Illing[51]	CR	ACEi-induced	1	na	na	+	na	+	na	+	-	na	na	Ineff.
Tran[52]	CR	Idiopathic	1	na	na	+/-	na	+	na	+	+/-	na	na	Ineff.

AE: angioedema. RCT: randomized controlled trial, CS: case series, CR: Case report, ACEi: angiotensin-converting-enzyme-inhibitor, na: not applicable, +: low risk of bias, - high risk of bias, +/- unclear risk of bias. *see also prophylactic setting table, Ineff: ineffective treatment described in the specific article.

TABLE 3: Risk of bias of prophylactic setting studies

Prophylactic setting				Selection bias			Performance bias		Detection bias			Attrition bias	Reporting bias	Remarks
Study	**Design**	**AE subtype**	**Usable sample size**	**Randomization**	**Allocation concealment**	**Case description**	**Blinding patient and personnel**	**Intervention**	**Blinding outcome**	**Assessment outcome**	**Adverse events**	**Incomplete outcome data**	**Selective reporting**	
Zazzali[53]	RCT	AE with wheals	208	+*	+*	na	+*	na	+*	na	na	+	+	*
Rijo Calderón[54]	Cohort	AE with wheals	10	na	na	+/-	na	+/-	na	+/-	+	na	na	
	Cohort	Idiopathic	4	na	na	+/-	na	+/-	na	+/-	+	na	na	
Mansi[19]	Cohort	Idiopathic	44	na	na	+	na	+	na	+	+	+	+	†
Wintenberger[55]	Cohort	Idiopathic	25	na	na	+	na	+	na	+	+	+	+	
Firinu[56]	Cohort	Idiopathic	16	na	na	+	na	+	na	+	+/-	+	+	
Saule[57]	Cohort	Idiopathic	20	na	na	+	na	+/-	na	+	+	na	na	
Du-Thanh[58]	CS	Idiopathic	25	na	na	+	na	+	na	+	+	na	na	
Cicardi[59]	CS	Idiopathic	15	na	na	+	na	+	na	+	+	na	na	
Azofra[60]	CS	Idiopathic	8	na	na	+	na	+	na	+	+	na	na	
Sands[61]	CS	Idiopathic	3	na	na	+	na	+	na	+	-	na	na	
vd Elzen[62]	CS	AE with wheals	3	na	na	+	na	+	na	+	+/-	na	na	
Groffik[63]	CS	AE with wheals	2	na	na	+	na	+	na	+	+/-	na	na	
Büyüköztürk[64]	CS	AE with wheals	1	na	na	+	na	+	na	+	+	na	na	
	CS	Idiopathic	2	na	na	+	na	+	na	+	+	na	na	
Perez[65]	CS	Idiopathic	2	na	na	+	na	+	na	+	+	na	na	
Ghazanfar[66]	CR	AE with wheals	1	na	na	+	na	+	na	+	+	na	na	
Wieder[67]	CR	AE with wheals	1	na	na	+	na	+	na	+	-	na	na	
Kutlu[68]	CR	AE with wheals	1	na	na	+	na	+	na	+	-	na	na	
Ozturk[69]	CR	AE with wheals	1	na	na	+	na	+	na	+	-	na	na	
Sánchez-Machín[70]	CR	AE with wheals	1	na	na	+	na	+	na	+	+	na	na	
Korkmaz[71]	CR	AE with wheals	1	na	na	+	na	+	na	+	-	na	na	
Stahl[43]	CR	Idiopathic	1	na	na	+	na	+	na	+	-	na	na	†
von Websky[72]	CR	Idiopathic	1	na	na	+	na	+	na	+	+	na	na	
Suna[73]	CR	Idiopathic	1	na	na	+	na	+	na	+	+	na	na	
Bayer[74]	CR	Idiopathic	1	na	na	+	na	+/-	na	-	-	na	na	
Vela Vizcaino[48]	CR	Idiopathic	1	na	na	+	na	+	na	+/-	-	na	na	†
Maggadottir[75]	CS	AE with wheals	2	na	na	+/-	na	+/-	na	+/-	-	na	na	Ineff

AE: angioedema. RCT: randomized controlled trial, CS: case series, CR: Case report, ACEi: angiotensin-converting-enzyme-inhibitor, na: not applicable, +: low risk of bias, - high risk of bias, +/- unclear risk of bias. *Study procedures described in separate articles †see also prophylactic setting table, Ineff: ineffective treatment described in the specific article.

Figure 1: Flowchart of in- and excluded articles

Treatment of acute attacks of AE

With regard to acute attacks of AE refractory to conventional treatment including antihistamines, corticosteroids, and adrenaline, the included articles described treatment of two subtypes: ACEi-AE and idiopathic AE.

ACEi-AE was addressed in 24 articles describing treatment of acute attacks in 154 patients, with study sizes varying from 1 to 58 patients. Outcome measures were (1) time to response (Fig. 2a and Table 4) and/or (2) proportion of patients with response (Fig. 2b and Table 4). As shown in Fig. 2a, described treatment strategies consisted of icatibant (42 patients in ten articles including one RCT) [5, 6, 21, 24, 26, 27, 31, 33, 36, 37], C1INH (14 patients in five articles) [22, 25, 28, 38, 39], FFP (13 patients in six articles) [23, 29, 30, 32, 35, 40], and kanokad (concentrate of vitamin K-dependent coagulation factor anti-vitamin K antagonist in one patient using anti-vitamin K medication concomitantly) [34]. In the 21 included studies for icatibant, C1INH, and FFP, the (median) time to initial response ranged from a few minutes up

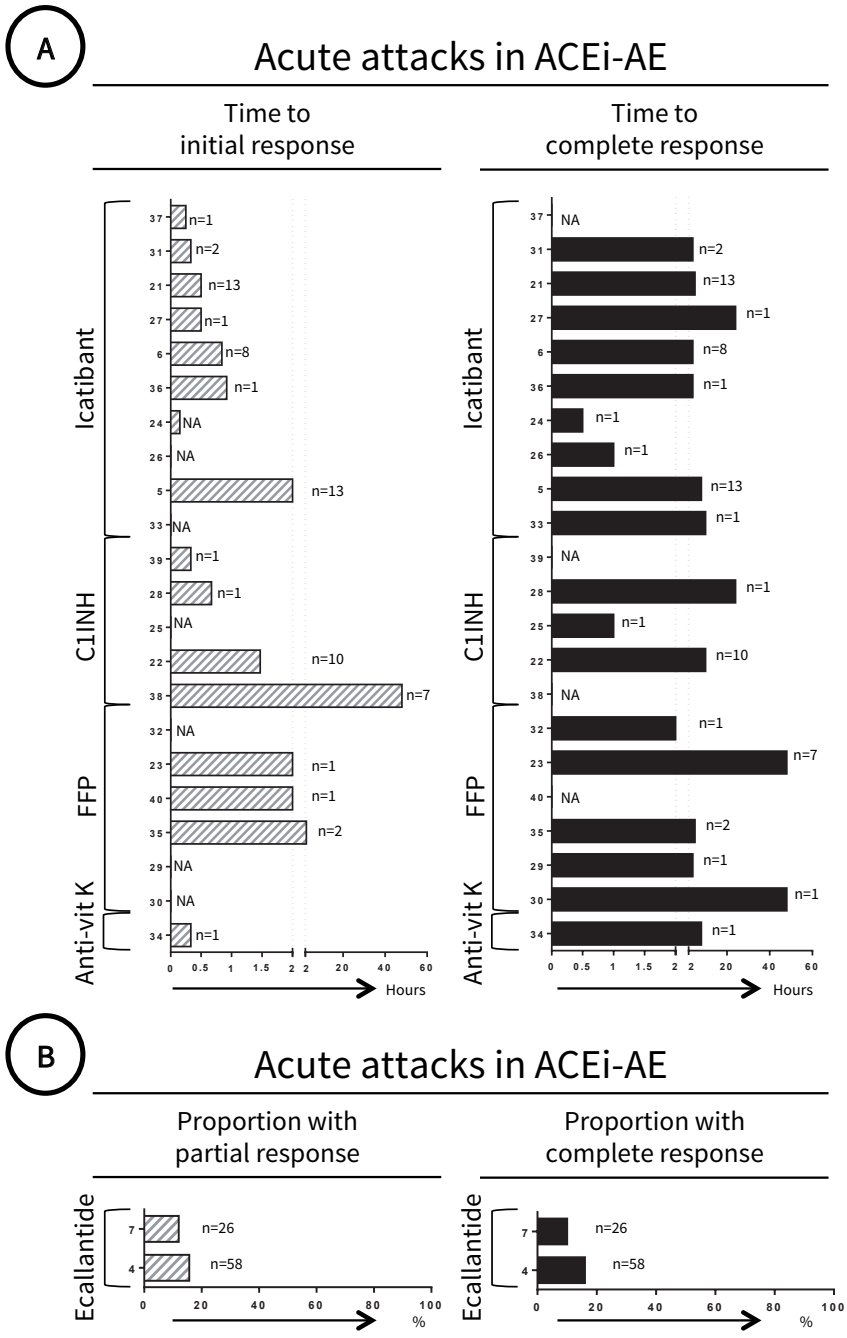
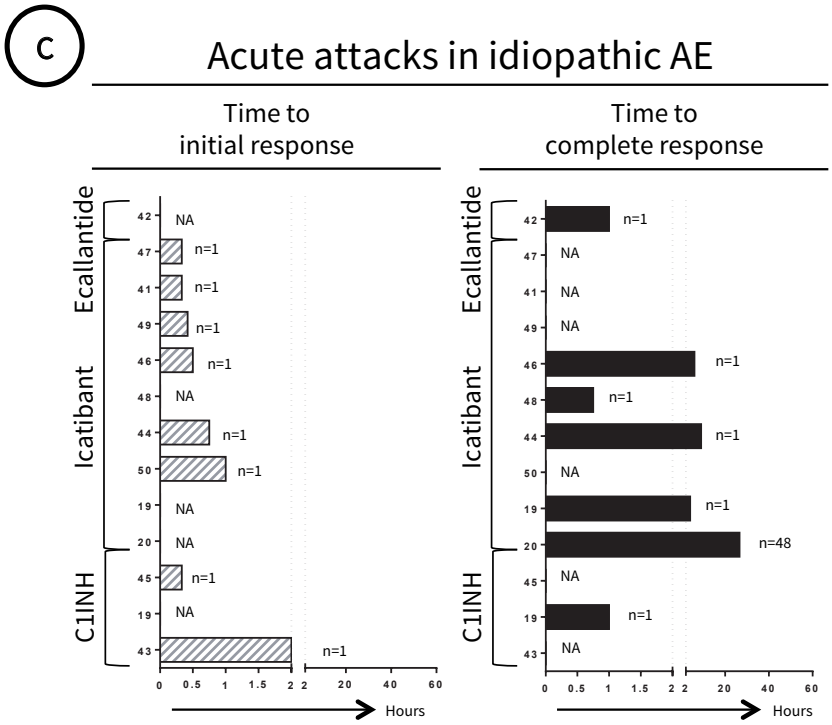


Figure 2: Response to treatment

NA: not available, Anti Vit K: anti vitamin K, C1INH: complement 1 esterase inhibitor, MTX: methotrexate, TA: tranexamic acid, P: progestin. Numbers on the Y-axis represent the reference number for each study, n indicates the number of patients included from each study. Not shown in figure 2c; Mansi et al, 13/24 patients had partial response to tranexamic acid. Not shown in figure 2d; Zazzali et al, in 208 patients treated with omalizumab, mean proportion of AE-free days was 90.1 to 95.8% versus 88.7% for placebo.

to 150 min, with one outlier up to 48 h [38]. Time to complete response ranged from 0.5 to 48 h. As shown in Fig. 2b, ecallantide was described additionally in 84 patients in two RCTs [4, 7]. Results for ecallantide were not significant: one RCT identified a difference in response rate vs. placebo of 16 % (95 % confidence interval, −11 to 41 %) [4], and a second RCT revealed a difference in response rate vs. placebo of 10 % (95 % confidence interval, −14 to 34 %) [7]. The level of evidence for C1INH, FFP, and icatibant was low, compared to ecallantide, due to lack of controlled studies. In conclusion, in treatment of acute attacks of ACEi-AE, no significant differences in the response rate between ecallantide and placebo were shown, and icatibant, C1INH, and FFP had similar times to response, mostly less than 2 h.

Idiopathic AE was addressed in 12 articles describing treatment of acute attacks in 84 patients. Effect of treatment was described as time to response (Fig. 2c and Table 5) or proportion of patients with response (Table 5). Treatment strategies consisted of icatibant (56 patients in nine studies) [19, 20, 41, 44, 46–50], TA (24 patients in one study) [19], C1INH (three patients in three articles) [19, 43, 45], and ecallantide (one patient) [42]. As shown in Fig. 2c, the time to initial response for C1INH ranged from 20 to 120 min and for icatibant from 20 to 45 min, and (median) time to complete response for ecallantide was 1 h. For C1INH, (median) time to complete response was also 1 h, and for icatibant this ranged from 45 min up to 26 h. In addition to Fig. 2c, one study reported response to TA in 13 of 24 patients (54 %) [19]. In conclusion, in acute attacks of idiopathic AE, C1INH, icatibant, and ecallantide had times to response often within 2 h, and TA was effective in more than 50 % of patients.



Prophylactic treatment of AE

With regard to recurrent AE refractory to conventional treatment, included articles about prophylactic treatment described two subtypes: AE with wheals, and idiopathic AE.

AE with wheals was addressed in 11 articles describing 230 patients. Effect was shown as time to response (Fig. 2d and Table 6) [53, 54, 62–64, 66–71]. All articles described treatment with omalizumab after unsuccessful treatment with antihistamines and often additional ineffective treatment options. One manuscript detailed two RCTs for which the results regarding urticaria had been published previously [10, 14]. However, in the included manuscript, specific results with regard to AE were described [53]. In the other articles, which consisted of cohort studies and case series or case reports, the time to initial effect ranged from 1 day to 60 days after administration, and 10 of 22 patients achieved complete remission within a time range varying from 1 day to <150 days [54, 62–64, 66–71]. In conclusion, in prophylactic treatment of AE with wheals, omalizumab had a broad range of time to response and was effective in almost half of the patients.

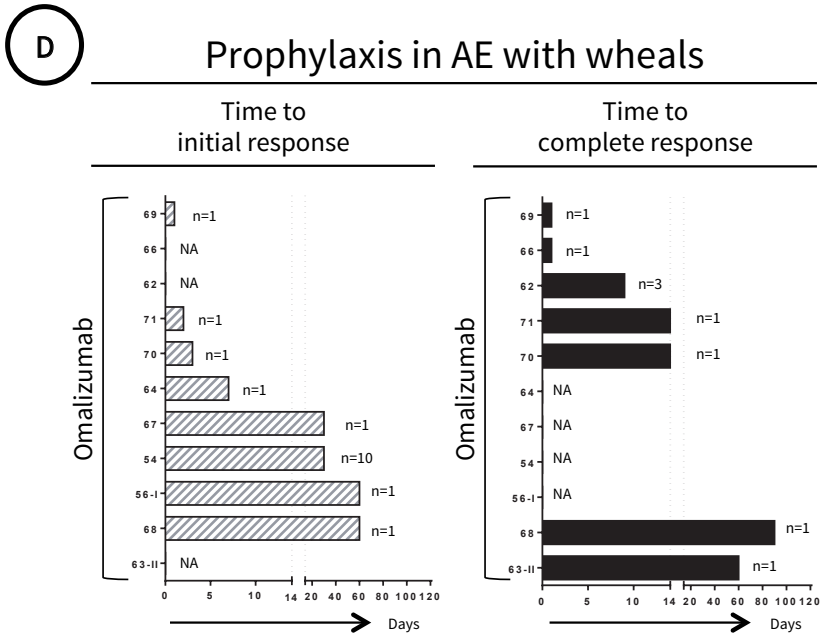


Figure 2 (continued)

Lastly, prophylactic treatment of idiopathic AE was addressed in 16 articles describing 168 patients [19, 43, 48, 54–61, 64, 65, 72–74]. Efficacy was shown as proportion of patients with response (Fig. 2e and Table 7) or time to response (Fig. 2f and Table 7). As shown in Fig. 2e, described treatment options were TA (126 patients in six studies) [19, 48, 55, 56, 58, 59],

progestin (20 patients in one study) [57], and C1INH (two patients in two studies) [43, 74]. When combining studies, TA led to improvement of symptoms in 92 patients (73 %) and a complete absence of symptoms in another 20 patients (16 %; Table 7). Progestin provided improvement in 19 of 20 patients and C1INH in two of two patients. Figure 2f shows the results for omalizumab (19 patients in six articles) [54, 60, 61, 64, 72, 73] and methotrexate (MTX, one patient) [65]. For omalizumab, in 12 patients (63 %), no further attacks occurred after starting treatment, and the time to initial response ranged from 1 day to 120 days. MTX provided improvement in one patient after 28 days of treatment. In conclusion, in prophylactic treatment of idiopathic AE, TA, omalizumab, and C1INH, as well as progestin and MTX, were effective in a majority of patients.

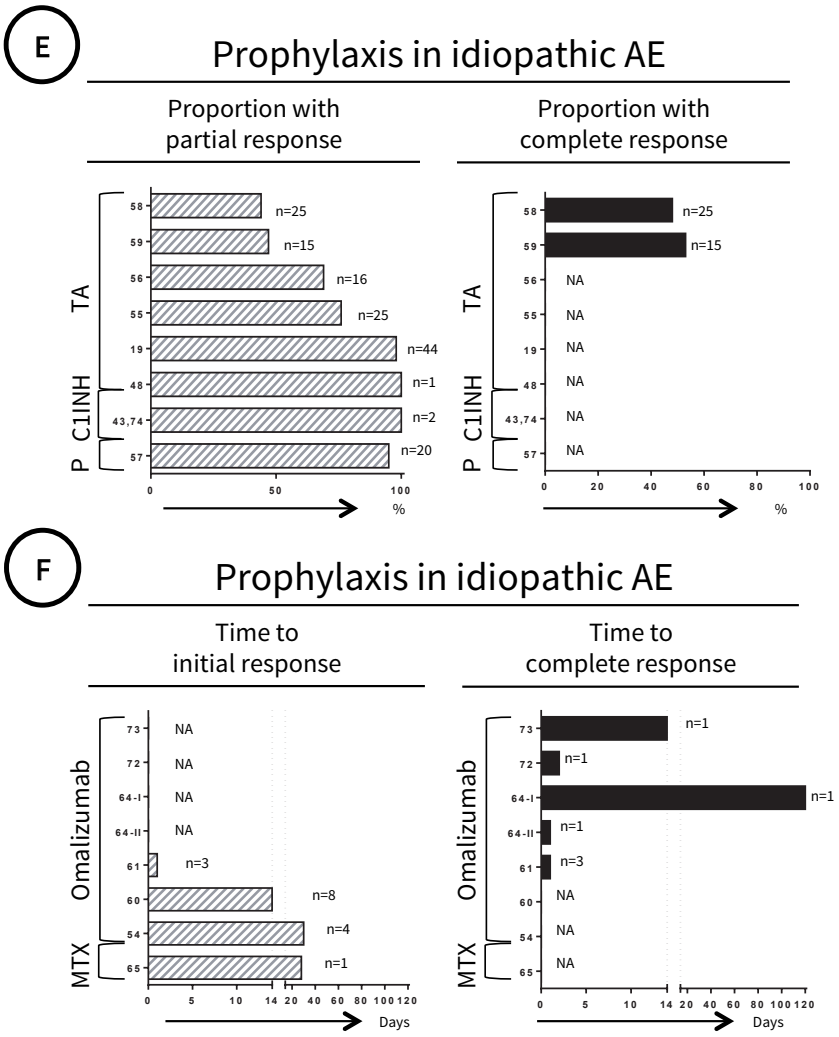


Figure 2 (continued)

TABLE 4: Results of acute setting studies: subtype ACEi–induced angioedema

Author	Year	Study design	Size	Previous therapy	Therapy	Dosage	Effect
Lewis[4]	2015	RCT	58+18	AH + C + E + H2	Ecallantide	10-60 mg	Predefined criteria ≤ 6hrs met in 88% versus 72% for PLC (difference 16%, 95%CI 11-41)
Bernstein[7]	2015	RCT	26+24	AH + C + E	Ecallantide	30 mg	Discharge criteria ≤ 4 hrs 31% versus 21% for PLC (difference 10%; 95% CI 14% to 34%)
Bas[5]	2015	RCT	13+14	None	Icatibant	30 mg	Median IR 120 min (95%CI 60-480) vs. 702 min (480-1080). CR 8.0 hrs vs. 27.1 hrs (3.0-16.0)
Bova[21]	2015	CS	13	AH + C + E	Icatibant	30 mg	IR 30 min (IQR 27.5-70). CR 5 hrs (IQR 4-7)
Bas[6]	2010	CS	8	None	Icatibant	30 mg	IR 50.6 min (SD 21). CR 4.4 hrs (SD 0.8)
Volans[31]	2013	CS	2	AH + C + E + TA	Icatibant	30 mg	IR 20 min. CR 4 hrs
Bartal[24]	2015	CR	1	AH + C + E + H2	Icatibant	30 mg	IR within minutes. CR 0.5 hrs
Charmillon[26]	2014	CR	1	n.r.	Icatibant	30 mg	CR 1 hr
Crooks[27]	2014	CR	1	AH + C + E	Icatibant	30 mg	IR 30 min. CR 24 hrs
Gallitelli[33]	2012	CR	1	None	Icatibant	30 mg	CR 10 hrs
Bas[36]	2011	CR	1	C	Icatibant	30 mg	IR 55 min. CR 4 hrs
Schmidt[37]	2010	CR	1	AH + C + E + C1INH	Icatibant	30 mg	IR 15 min
Greve[22]	2014	CS	10	None	C1INH (B)	1000 U	IR 88 min (SD 38). CR 10.1 hrs (SD 3)
Lipski[25]	2015	CR	1	C + E + FFP	C1INH (B)	20 U/kg	CR <1 hr
Rasmussen[28]	2014	CR	1	None	C1INH	15 U/kg	IR 40 min. CR <24 hrs
Dehne[38]	2007	CR	1	AH + C + E + P + FFP	C1INH (B)	1000 IE	IR 2 days after initial worsening in the first 24 hrs
Nielsen[39]	2006	CR	1	AH + C	C1INH (B)	1500 U	IR 20 min.
Hassen[23]	2013	CS	7	AH + C + E + H2	FFP	1-3 U	IR 2 hrs. CR 48 hrs
Stewart[35]	2012	CR	2	C	FFP	2 U	IR 2.5 hrs in 1 patient. CR 4.75 in the other
Yates[29]	2014	CR	1	None	FFP	2 U	CR 4 hrs
Bledsoe[30]	2013	CR	1	AH + C + E + H2	FFP	2 U	IR within few hrs. CR < 48 hrs
Bolton[32]	2012	CR	1	Not known	FFP	2 U	CR 2 hrs
Karim[40]	2002	CR	1	AH + C	FFP	4 U	IR <2 hrs
Millot[34]	2012	CR	1	AH + C + E	Kanokad	1500 U	IR 20 min. CR 8 hrs

CS: case series, CR: Case report, ACEi: angiotensin-converting-enzyme-inhibitor, n.r.: not reported. AH: antihistamine, C: corticosteroids, E: epinephrine, C1INH: C1 inhibitor concentrate, (B: Berinert P), TA: tranexamic acid, H2: H2 antagonist, FFP: fresh frozen plasma, P: pantoprazole, PLC: placebo. Size: only the number of patients included for describing therapies are presented in the table. In controlled studies the number of patients treated with study medication, versus those treated with placebo or comparative treatment, are presented as x+y. The effect of treatment is presented as IR: initial response, and CR; complete response.

TABLE 5: Results of acute setting studies: subtype idiopathic angioedema

Author	Year	Study design	Size	Previous therapy	Therapy	Dosage	Effect
Bouillet[20]	2014	Cohort	48	Unknown	Icatibant	n.r.	Median time to CR 26,6 hrs (IQR 8,3-46)
Bertazzoni[41]	2015	CR	1	AH + C + E	Icatibant	30 mg	IR 20 min
Seoane[50]	2014	CR	1	AH + C	Icatibant	n.r.	"rapid response"
Vela Vizcaino[48]	2014	CR	1	AH + C + E	Icatibant	n.r.	CR 45 min
Montinaro[44]	2013	CR	1	AH + C	Icatibant	30 mg	IR 45 min. CR 9 hrs
Colás[49]	2012	CR	1	AH + C + E + H2 + C1INH	Icatibant	30 mg	IR 25 min.
Lleonart[46]	2012	CR	1	AH + C + E	Icatibant	30 mg	IR 30 min. CR 6 hrs
Sridhara[47]	2012	CR	1	AH + C + E + H2 + H + LTRA	Icatibant	30 mg	IR 20 min
	2014	Cohort	1	None	Icatibant	30 mg	CR 4 hrs
Mansi[19]	2014	Cohort	24	None	TA	≤ 6g/day	Decreased severity and duration of symptoms in 13 (54%)
	2014	Cohort	1	None	C1INH	1000 U	CR 1 hr
Stahl[43]	2014	CR	1	AH + C + E + H2 + TA + H + AB + LTRA + FFP + Icatibant	C1INH	20 U/kg	IR 2 hrs
O'Keefe[45]	2013	CR	1	C + E	C1INH	500 U	IR 20 min
Nanda[42]	2014	CR	1	AH + C + E	Ecallantide	30 mg	CR <1 hr

CS: case series, CR: Case report, n.r.: not reported. AH: antihistamine, C: corticosteroids, E: epinephrine, C1INH: C1 inhibitor concentrate, TA: tranexamic acid, H2: H2 antagonist, FFP: fresh frozen plasma, LTRA: leukotriene receptor antagonist, H: hormones, AB: antibiotics, H: hydroxychloroquine. The effect of treatment is presented as IR: initial response, and CR; complete response.

TABLE 6: Results of prophylactic setting studies: subtype angioedema with wheals

Author	Year	Study design	Size	Disease duration (years)	Previous therapy	Therapy	Dosage scheme	Effect	Follow up (months)
Zazzali[53]	2014	RCT	208	n.r.	AH	OMA	75-300/4	Mean proportion AE-free days 90.1 to 95.8%, versus 88.7%	3
Rijo Calderón[54]	2013	Cohort	10	n.r.	AH + C + dapsone	OMA	150-300/2-4	No further attacks in 5, mild symptoms in 7*	n.r.
vd Elzen[62]	2014	CS	3	2,4,9	AH + C + LTRA + H2 + AB + I + MTX + HC	OMA	150-300/2-4	CR < 9 days	24
Groffik[63]	2010	CS-1	1	1	AH + C + LTRA	OMA	300/2	IR < 2 months	4
		CS-2	1	19	AH + C + LTRA	OMA	150/2	CR < 2 months	4
Büyüköztürk[64]	2012	CS	1	7	AH	OMA	225/4	IR <1 week	8
Ghazanfar[66]	2015	CR	1	n.r.	AH + C + I	OMA	150/2 → 300/4	CR < 1 day	48
Wieder[67]	2015	CR	1	n.r.	AH + C + LTRA + I + O	OMA	300/4	IR After first dose	29
Kutlu[68]	2014	CR	1	n.r.	AH + C + E	OMA	300/4	IR 2 months. CR 3 months	3
Ozturk[69]	2014	CR	1	n.r.	AH + C + O	OMA	300/4	No further attacks	3
Sánchez-Machín[70]	2011	CR	1	9	AH + C + I + O	OMA	300/2→ 300/6	IR <3 days. CR 14 days	36
Korkmaz[71]	2010	CR	1	n.r.	AH + C + LTRA + H2 + AB + I	OMA	300/2	IR 2 days. CR 14 days	n.r.

CS: case series, CR: Case report, n.r.: not reported. AH: antihistamine, C: corticosteroids, E: epinephrine, C1-inh: C1 inhibitor concentrate, TA: tranexamic acid, H2: H2 antagonist, LTRA: leukotriene receptor antagonist, AB: antibiotics, I: other immunosuppressant, MTX: methotrexate, HC: hydroxychloroquine, O: other therapy. Dosage scheme presented as mg administered every x weeks, an arrow indicates a dose adjustment during the treatment period. The effect of treatment is presented as IR: initial response, and CR; complete response. *Results mentioned for the whole study population, which may be larger than the patients included in this review.

TABLE 7: Results of prophylactic setting studies: subtype idiopathic angioedema

Author	Year	Study design	Size	Disease duration (years)	Previous therapy	Therapy	Dosage scheme	Effect	Follow up (months)
Mansi[19]	2014	Cohort	44	n.r	n.r.	TA	3g/day → 0.5 - 3 mg/day	Reduction recurrences in 43 (98%)	n.r.
Wintenberger[55]	2014	Cohort	25	n.r.	n.r.	TA	2 - 2.5 g/day	Attack frequency from 15.2 (range 2-50) to 3.7 (0-18) per 6 months. No response in 6 (24%).	6
Firinu[56]unknown genetic defect (U-HAE	2015	Cohort	16	n.r.	AH + C	TA	1.5 - 3 g/day	50% attack frequency decrease in 8 (50%), no response in 5 (31%), other in 3.	n.r.
Du-Thanh[58]	2010	CS	25	n.r.	AH + C	TA	3 g/day	CR in 12 (48%), PR in 11 (44%), no response in 2 (8%)	20
Cicardi[59]	1999	CS	15	Median 6	AH	TA	3 g/day	No further attacks in 8 (53%), 7 attack frequency decreased by ≥ 75%	10-282
Vela Vizcaino[48]	2014	CR	1	3	AH + C + E + C1INH	TA	3 g/day	Attack frequency decrease from weekly to 3 /8 wk	n.r.
Saule[57]	2012	Cohort	20	n.r.	AH	Progestin	n.r.	Improvement in 19 (95%)	32,4
Rijo Calderón[54]	2013	Cohort	4	n.r.	AH + C + dapsone	OMA	150-300/2-4	IR <1 month	n.r.
Azofra[60]	2015	CS	8	n.r.	(AH + C +) TA	OMA	300/4	IR 2-14 days	6-12 m
Sands[61]	2007	CS-1	1	6	AH + C + E + H2	OMA	300/3	No further attacks	24
		CS-2	1	4	AH + C + H2	OMA	375/2	No further attacks	7
		CS-3	1	9	AH + C + LTRA + H2	OMA	300/4	1 minor attack in 2 yrs	> 12
Büyüköztürk[64]	2012	CS-1	1	10	AH + C + H+ O	OMA	300/4	CR within 4 months	n.r.
		CS-2	1	15	AH + C + H + IVIG + I	OMA	300/4	No further attacks	n.r.
von Websky[72]	2013	CR	1	n.r.	AH + C + LTRA + AB	OMA	300/4	CR 2 days	18
Suna[73]	2009	CR	1	19	AH + C + H + IVIG + I	OMA	300/2	CR <14 days	4,5
Stahl[43]	2014	CR	1	1	AH + C + E + H2 + TA + H + AB + LTRA + FFP + Ica	C1INH	1000 U/ twice weekly	Attack frequency decrease 5-7/month to 1.5/month	n.r.
Bayer[74]	2013	CR	1	n.r.	AH + C + E + H2 + LTRA + H + I	C1INH	n.r.	Improvement after 2 doses of C1INH	n.r.
Perez[65]	2010	CS-1	1	2.75	AH + C + I	MTX	15/1	IR 28 days	n.r.

CS: case series, CR: Case report, n.r.: not reported. AH: antihistamine, C: corticosteroids, E: epinephrine, C1-inh: C1 inhibitor concentrate, TA: tranexamic acid, H2: H2 antagonist, FFP: fresh frozen plasma, P: pantoprazole, LTRA: leukotriene receptor antagonist, H: hormones, AB: antibiotics, I: immunosuppressant, MTX: methotrexate, H: hydroxychloroquine, IVIG: intravenous immunoglobulin, Ica: Icatibant, O: others. Dosage scheme presented as mg administered every x weeks, unless stated otherwise. The effect of treatment is presented as IR: initial response, CR; complete response, and PR: partial response.

Ineffective treatment options

Ineffective treatment options were described in 21 patients in 12 articles (Table 8) [25, 31, 37, 38, 43, 48, 49, 51, 52, 60, 62, 75]. Nine of them overlap with the previously described articles since they had additionally described a successful treatment option for at least one of the subtypes of AE [25, 31, 37, 38, 43, 48, 49, 60, 62]. In three articles, only ineffective treatment options were described [51, 52, 75]. In total, ineffectiveness was recorded for TA (12 patients), C1INH and FFP (five patients each), and icatibant, MTX, and omalizumab (two patients each). In four patients, more than one therapy was recorded ineffective, in addition to conservative treatment with antihistamines, corticosteroids, and/or adrenaline. In conclusion, ineffectiveness was reported for several therapeutic options commonly used in bradykinin-mediated and mast cell-mediated AE and was reported for individual cases only, resulting in low numbers for each drug.

TABLE 8: Results of articles describing ineffective treatment

Author	Year	AE subtype	Study design	Size	Previous therapy	Ineffective therapy	Dosage
Articles describing ineffective and effective treatments:							
Volans[31]	2013	ACEi-AE	CS	2	AH + C + E	TA	
Schmidt[37]	2010	ACEi-AE	CR	1	AH + C + E	C1INH	
Lipski[25]	2015	ACEi-AE	CR	1	C + E	FFP	
Dehne[38]	2007	ACEi-AE	CR	1	AH + C + E + P	FFP	
Colás[49]	2012	Idiop. (acute)	CR	1	AH + C + E + H2	C1INH	
Stahl[43]	2014	Idiop. (acute and proph.)	CR	1	AH + C + E + H2 + H + AB + LTRA	TA Icatibant FFP	
vd Elzen[62]	2014	AE with wheals	CS	1	AH + LTRA + I	MTX	n.r.
Vela Vizcaino[48]	2014	Idiopathic (acute + proph)	CR	1	AH + C + E	C1INH	
Azofra[60]	2015	Idiop. (proph)	CS	8	AH + C, or none	TA	
Articles describing ONLY ineffective treatments:							
Illing[51]	2012	ACEi-AE	CR	1	AH + C + E	Icatibant	30 mg
Tran[52]	2013	Idiop (acute)	CR	1	AH + C	FFP TA C1INH*	n.r.
Maggadottir[75]	2013	AE with wheals	CS-1	1	AH + LTRA + TCA + AB + MTX	OMA MTX†	
	2013	AE with wheals	CS-2	1	AH + C + LTRA + IVIG + I	FFP OMA C1INH	

Idiop: idiopathic, Proph: prophylactic, CS: case series, CR: Case report, CS-x: patient number x in the specific case series, n.r.: not reported. AH: antihistamine, C: corticosteroids, E: epinephrine, C1-INH: C1 inhibitor concentrate, TA: tranexamic acid, H2: H2 antagonist, FFP: fresh frozen plasma, P: pantoprazole, LTRA: leukotriene receptor antagonist, H: hormones, AB: antibiotics, I: immunosuppressant, MTX: methotrexate, IVIG: intravenous immunoglobulin, Ica: Icatibant, TCA: tricyclic antidepressant. *Icatibant effective, not included due to insufficient details. †IVIG effective, not included due to insufficient details.

Safety

The presence or absence of adverse effects was addressed in 25 of the 61 included articles [4–7, 19, 21, 22, 27, 42, 46, 54–60, 62–66, 70, 72, 73] (Table 9). The other 36 articles did not report information on this topic. Thus, safety information was available for 315 patients treated with either ecallantide (87 patients), icatibant (37 patients), TA (125 patients), omalizumab (34 patients), progestin (20 patients), C1INH (ten patients), or MTX (two patients).

A distinction between SAEs and less severe TEAEs was adopted from the included articles, if available. Additionally, only adverse events possibly, probably, or definitely related to treatment are shown in this review. SAEs were reported in six patients, including five AE episodes during treatment with ecallantide (6 % of those treated with ecallantide) and one myocardial infarction during treatment with TA (0.8 %). TEAEs were reported in 13 patients treated with ecallantide (15 %) and 12 treated with icatibant (32 %; all local and related to the administration method). At least 19 patients treated with TA (15 %) reported TEAE, and at least nine treated with omalizumab (26 %). However, TEAEs were presented in the total study populations including also HAE and CSU patients; therefore, the number of patients experiencing adverse effects may be higher. For progestin and MTX, TEAEs were addressed in one article each, where TEAEs were also presented in the total study population including also HAE and CSU patients. For C1INH, no TEAE was reported. In addition, one article described the use of omalizumab during two pregnancies, with no developmental abnormalities in both children [66]. In conclusion, SAEs were reported in 2 % and TEAE in 17 % of patients.

DISCUSSION

In this systematic review we found several treatment options for patients with refractory AE. For acute attacks of AE, several articles described treatment with icatibant, C1INH, TA, FFP, and ecallantide. For prophylactic treatment of AE omalizumab, TA, and C1INH were shown effective, and with fewer included articles also progestin and MTX. The described treatments showed a good efficacy in addition to a favorable safety profile with a low number of mostly mild and self-limiting adverse effects. A limitation of the available literature was the low level of evidence for all treatment options except ecallantide and icatibant.

In ACEi-AE, high-quality studies were performed for ecallantide but response rates compared to placebo were not significant. Many patients responded quickly after treatment with icatibant, C1INH and TA, but most of the included studies were not controlled and therefore of lower quality in terms of scientific reliability. FFP has shown similar results, but since FFP also contains other substrates including prekallikrein and high molecular weight kininogen, it has been hypothesized to have the potential to worsen an acute attack of AE since new bradykinin can be formed [76]. Treatment of refractory ACEi-AE mostly consisted of drugs known for treatment of HAE. The rationale for this is that ACEi-AE is presumably bradykinin-mediated [2]. Icatibant had a similar time to response in ACEi-AE as previously shown in HAE patients

TABLE 9: (Serious) treatment–emergent adverse events reported per treatment option

Study	Angioedema subtype	Sample size	Therapy	SAEs	Number of pts with ≥1 TEAE	TEAEs
Lewis[4]	ACEi-induced	58+18	Ecallantide	5 related SAEs (8.9%; all angioedema). One death in placebo group due to respiratory compromise.	30 (51.7%) vs. 8 (44.4%); 13/30 related	AE (20 cases); headache and hypoesthesia (2 cases each); abdominal pain, diarrhea, hematuria, injection site pain, injection site swelling, muscle spasms, oropharyngeal pain, oral candidiasis, pain in extremity, and pruritic rash (1 case each).
Bernstein[7]	ACEi-induced	26+24	Ecallantide	2 (7.7%) versus 6 (25%), none related	18 (75%) vs. 17 (65.4%); none related	n.a.
Nanda[42]	Idiopathic	1	Ecallantide	0	0	n.a.
Bas[5]	ACEi-induced	13+14	Icatibant	0 v.s. 1 (7%)	1 (7%) vs. 4 (27%); 1/1 related	patient-reported injection site pain; additional investigator-assessed injection site reactions in ≥ 12 (80%)
Bova[21]	ACEi-induced	13	Icatibant	n.r.	1	injection site pain
Bas[6]	ACEi-induced	8	Icatibant	0	8	injection site erythema and/or itching
Crooks[27]	ACEi-induced	1	Icatibant	0	1	injection site erythema
Lleonart[46]	Idiopathic	1	Icatibant	0	1	injection site pain
Mansi[19]	Idiopathic	44	TA	n.r.	5	Migraine, menstrual irregularities, dyspepsia, diarrhea
Wintenberger[55]	Idiopathic	25	TA	0	11	Abdominal pain, dizziness, weakness, pain in lower limbs, migraine
Du-Thanh[58]	Idiopathic	25	TA	n.r.	1	Digestive intolerance
Firinu[56]	Idiopathic	16	TA	n.r.	Unclear*	Abdominal discomfort and migraine (1 case), abdominal discomfort (unclear)
Cicardi[59]	Idiopathic	15	TA	1 (myocardial infarction after 11 months of treatment)	2	Laryngeal/pharyngeal dryness, self-limiting in months

Related denote possibly, probably or definately related to study drug, as described in the separate articles. The number of TEAEs may be higher than the number of patients reporting TEAEs since patients may have reported more than one TEAE. For RCTs, the sample size is shown as the number of treated patients + patients treated with placebo. SAE and TEAE are only shown as recorded for the treatment groups

n.a.: not applicable since patients had reported no adverse effects, or all adverse events were unrelated to the study medication

*TEAEs mentioned for the whole study population, which may be larger than the patients included in this review, e.g. in case of chronic spontaneous urticaria with or without angioedema

TABLE 9 (continued)

Study	Angioedema subtype	Sample size	Therapy	SAEs	Number of pts with ≥1 TEAE	TEAEs
Rijo Calderón[54]	AE with wheals	10	OMA	n.r.	7*	drowsiness (n=7), digestive, cutaneous symptoms, and weight loss (5)
Azofra[60]	Idiopathic	8	OMA	0	0	n.a.
Rijo Calderón[54]	Idiopathic	4	OMA	n.r.	7*	drowsiness (n=7), digestive, cutaneous symptoms, and weight loss (5)
vd Elzen[62]	AE with wheals	3	OMA	0	2	headache in patient co-treated with ciclosporin, malaise (1 case each)
Büyüköztürk[64]	Idiopathic	2	OMA	0	0	n.a.
Groffik[63]	AE with wheals	2	OMA	0	3/9*	headache, blood pressure decrease, fatigue; self-limiting 3-4 days after first 3 injections
Büyüköztürk[64]	AE with wheals	1	OMA	0	0	n.a.
Ghazanfar[66]	AE with wheals	1	OMA	0	0	n.a.
Sánchez-Machín[70]	AE with wheals	1	OMA	0	0	n.a.
von Websky[72]	Idiopathic	1	OMA	0	0	n.a.
Suna[73]	Idiopathic	1	OMA	0	0	n.a.
Saule[57]	Idiopathic	20	Progestin	0	17/55*	weight gain (5 cases), oestrogenic deficiency (4), breakthrough bleeding (2), hyperandrogenia (2), n.r. (4)
Greve[22]	ACEi-induced	10	C1INH (B)	0	0	n.a.
Perez[65]	Idiopathic	2	MTX	1, unrelated	unclear*	hair thinning and fatigue

[77]. Ecallantide had stronger beneficial results in HAE patients [78] compared to ACEi-AE patients, partially due to a high response rate in the placebo group. Additional RCTs in HAE patients revealed time to onset of relief within 2 hours for pasteurized C1INH, nanofiltered C1INH, and recombinant human C1INH (rhC1INH) [79–81]. In many of the cases included in this review, the onset of relief after C1INH was reported within one hour of administration, and efficacy results may therefore be quite consistent with the results of C1INH treatment in acute HAE attacks. Very recently, the Canadian Agency for Drugs and Technologies in Health performed a non-systematic literature search and provided a summary of four available guidelines for urticaria and AE, which supports that icatibant, C1INH, ecallantide, and FFP may be useful in treatment of ACEi-AE [82]. In addition to results of these therapies in ACEi-AE, we show in the current review that these therapies, with icatibant as most often studied, may also be effective in treatment of acute attacks of idiopathic AE. In conclusion, in patients suffering ACEi-AE or an acute attack of idiopathic AE, ecallantide seems to have effect in a limited number of patients, if any, whereas icatibant, C1INH, TA, and FFP often lead to symptom relief within 2 hours in addition to a good safety profile.

For AE with wheals, also known as CSU, omalizumab was the only treatment option described when conservative treatment had failed. A high success rate, good safety profile, and rapid responses were described, as was shown extensively in patients suffering CSU, which by definition includes AE with wheals [1, 10–15]. In patients suffering idiopathic AE, we show that both licensed HAE drugs and omalizumab seem to have a beneficial effect in a substantial amount of patients, even in those who are very refractory and have had many other treatments prior to the described treatment. When comparing with ACEi-AE, it appears that idiopathic AE responds even more rapidly upon treatment with icatibant, C1INH or ecallantide. This suggests a role for both bradykinin and mast cells (histamine) in idiopathic AE with normal C1INH, although this was not the objective of the current review. Additionally, in 1 patient treated with C1INH, and 2 treated with FFP, the time to response of an acute attack was reported to be 2 days [30]. In such cases one should be aware of the natural course of an attack [1–3, 83]. Furthermore, also for this subtype, the level of evidence is low, and controlled studies remain to be performed. In conclusion, omalizumab, TA, and C1INH were effective and safe in a majority of patients in need for prophylactic treatment of refractory idiopathic AE or AE with wheals.

One needs to keep in mind that all treatment options described are currently off-label in these patient groups worldwide, except for omalizumab in AE with wheals (CSU), and that the findings should be confirmed in clinical trials. Due to the fact that most therapies described have only been registered for other indications recently, efficacy and safety for the current subtypes of non-HAE have not been studied yet. It remains unclear which (groups of) patients derive a beneficial effect from each type of treatment. For C1INH, a beneficial effect was described even in patients who failed to respond to icatibant and/or FFP. On the contrary, in

ACEi-AE and idiopathic AE patients failed to respond to C1INH, but did respond to icatibant or TA. Similar results were seen for TA, FFP, icatibant, MTX, and omalizumab, indicating the presence of non-responders for each type of treatment in almost each subtype of AE, and also indicating that switching treatment options can lead to satisfactory results in some individuals even when both target a similar pathophysiological mechanism.

We opted for a broad overview of the level of evidence of treatment options when performing this systematic review. This was deemed appropriate with regard to the research question, and the therapeutic problems physicians face in daily practice. The results may be an overestimation since case reports generally represent one or few patients with positive effects of treatment, and only few cases without response are available possibly due to underreporting. Due to the use of different outcome measures, such as percentage of patients with response, or the time to response, it was difficult to compare results of the studies. Additionally, we found there to be a low level of prior research evidence. Fortunately, in the last couple of years more extensive research has been published, allowing for the inclusion of several RCTs in this review. Still, our results illustrate the need for further research in these patient groups including prospective cohort studies and controlled studies. The lack of available guidelines underlines this further. Not included in this review but worthy of mention is the fact that it is known that AE is known to have a detrimental effect on quality of life (QoL) [84]. While the impact on the QoL was not a part of this review, is it striking that this aspect was not addressed in many of the included studies. Disease-specific questionnaires have been developed for AE patients, both with regard to disease activity and QoL [1, 84–86], and we consider QoL an important additional outcome measure both in acute attacks and prophylactic setting studies.

A minority of articles included information with respect to adverse effects of treatment. When reported, only few patients experienced adverse effects. These were generally mild and self-limiting, and most were known side effects [15, 76, 87–91]. New TEAE were oropharyngeal discomfort (reported for TA), weight loss (omalizumab), and hypoesthesia, hematuria, muscle spasms, oral candidiasis, and pain in extremity (ecallantide, TEAEs may be unrelated). Notably, for icatibant only injection-related TEAEs occurred.

In conclusion, for patients suffering angioedema refractory to conservative treatment, several additional treatment options are available with a rapid time to response, high response rates, and limited side effects. However, these therapies are off-label and there is a need for additional studies to provide a high level of scientific evidence. Treatment options differ per subtype of AE. Most promising treatments for acute attacks (ACEi-AE and idiopathic AE) consist of icatibant, C1INH, and FFP with response often within 2 hours and with limited side effects. For prophylactic treatment (idiopathic AE and AE with wheals) most promising

options are omalizumab, TA, and C1INH, with efficacy in a majority of patients, together with limited side effects.

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CHAPTER 4:

ALLERGENICITY AND SAFETY OF RECOMBINANT HUMAN C1 ESTERASE INHIBITOR IN PATIENTS WITH ALLERGY TO RABBIT OR COW'S MILK

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ABSTRACT

Background

Recombinant human C1 inhibitor (rhC1INH) for on demand treatment of hereditary angioedema, is purified from milk of transgenic rabbits. It contains low amounts (<0.002%) of host-related impurities (HRI), which could trigger hypersensitivity reactions in patients with rabbit allergy (RA) and/or cow's milk allergy (CMA).

Objective

Assessment of allergenicity and safety of rhC1INH in RA and/or CMA patients.

Methods

Patients with CMA and/or RA underwent skin prick test (SPT), intracutaneous test (ICT), and, when both were negative, subcutaneous (SC) challenge with up to 2100U (14 mL) rhC1INH. The negative predictive value (NPV) of the skin test protocol was calculated, defined as the ratio of patients without systemic symptoms of hypersensitivity following SC challenge, over the number of patients having tested negative for both the SPT and ICT. Adverse events after exposure to rhC1INH were recorded.

Results

Twenty-six patients with RA and/or CMA were enrolled. Twenty-four had negative SPT and ICT for rhC1INH, whereas 2 had negative SPT, but positive ICT to rhC1INH (only the highest concentration). Twenty-two patients with negative SPT and ICT underwent SC challenge. None developed allergic symptoms. Local treatment-emergent adverse events (TEAEs) occurred in 7 patients (32%) after SC challenge. In 5 these were considered drug related. All were mild.

Conclusion

None of the patients with negative SPT and ICT for rhC1INH had allergic symptoms during rhC1INH challenge. The NPV of the combination of SPT and ICT for the outcome of the SC challenge was 100% (95% CI: 84.6% ; 100%). SC administration of rhC1INH was well-tolerated.

INTRODUCTION

Acute attacks of hereditary angioedema (HAE) due to C1 esterase inhibitor (C1INH) deficiency (C1INH-HAE) can be treated by replacing C1INH. Several C1INH drugs are approved for this indication, including recombinant human C1INH (rhC1INH, conestat alfa, Ruconest).[1] The active constituent rhC1INH is secreted in the milk of transgenic New Zealand White rabbits expressing the human gene for C1INH , and has an amino-acid sequence identical to that of endogenous human C1INH.[2–4] rhC1INH is a highly-purified final product which contains <0.002% of host-related impurities (HRI)[2,3,5] consisting primarily of milk or dander proteins. It is unclear whether these impurities are able to trigger allergic reactions in patients with a pre-existing allergy to rabbit dander exposed to rhC1INH. Additionally, although cow's milk proteins differ from rabbit milk proteins, rabbit milk impurities in rhC1INH may theoretically elicit an allergic reaction in patients with cow's milk allergy (CMA) due to cross-reactivity of involved IgE with HRIs in rhC1INH.[3]

rhC1INH is contraindicated in patients with known or suspected allergy to rabbits (labeling EU) or rabbit-derived products (labeling USA). This is largely based on the occurrence of a single allergic reaction in a healthy study subject with a previously undisclosed rabbit allergy in a safety study conducted in 2005.[3] This study subject developed an itchy rash and wheezing upon first exposure to 100 U/kg rhC1INH shortly after the start of the intravenous infusion. Subsequently, an elevated anti-rabbit epithelium IgE value of 39.6 kU/L was found. Several HAE patients included in the clinical program with rhC1INH who also had elevated anti-rabbit IgE did not develop allergic reactions to rhC1INH, including after repeated exposure.[3]

To further investigate whether rhC1INH is safe in subjects with rabbit allergy (RA) or CMA, we used a skin test protocol to assess the allergic potential of rhC1INH in patients with RA and/or CMA without C1INH deficiency. The primary objective of the study was to assess the allergenicity of rhC1INH, using the negative predictive value (NPV) of a combination of skin prick test (SPT) and intracutaneous test (ICT) for development of allergic reactions during a subcutaneous (SC) challenge with rhC1INH in patients with clinical RA and/or CMA. Furthermore, the safety of the SC challenge was assessed.

METHODS

A prospective study was carried out to evaluate the allergenicity and safety of rhC1INH in patients with RA and/or CMA.

Study population

Patients with an age between 18 and 65 years, with clinically confirmed RA and/or CMA were contacted for participation in the study. RA and CMA were defined as a suggestive history with acute symptoms after exposure to rabbit or after ingestion of cow's milk respectively, in combination with sensitization by positive SPT or by the presence of serum IgE > 0.35

kU/L for rabbit dander in case of RA, or for cow's milk extract or for at least one of the known cow's milk allergens in case of CMA. IgE and SPT results were calculated only for those with a positive SPT to the specific allergen. In case of CMA, diagnosis could additionally be confirmed by double-blind placebo controlled food challenge (DBPCFC) as part of standard care, prior to study inclusion. Main exclusion criteria were the presence of severe dermographism, and pregnancy. In female participants with childbearing potential, a urine pregnancy test was performed.

This study was approved by the local Ethics committee (UMC Utrecht, protocol number 11-345), written informed consent was obtained from all participants.

Skin testing and order of proceedings

To assess the allergenicity of rhC1INH, a skin test protocol was carried out. The skin test protocol consisted of three subsequent types of skin testing with increasing dosages of rhC1INH: a SPT, ICT, and SC challenge. Patients could only proceed to the next skin test when the previous test was negative. Antihistamines or other drugs that could interfere with skin testing were temporarily discontinued according to predefined washout periods including but not limited to 7 days for systemic or topical steroids, and 4 days for most antihistamines. All SPT and ICT tests were negatively and positively controlled with saline and histamine, respectively. rhC1INH was diluted with water for injection. All tests were performed with an observation period of up to 2 hours between each injection, and with an observation time up to 3 hours before discharge. All patients were contacted by phone by the site personnel within 24 hours after all exposures to rhC1INH to enquire of any adverse effects.

Each test started with the lowest concentration or volume of rhC1INH before proceeding to higher concentrations or volumes. Firstly, a SPT with 1:10 and undiluted rhC1INH was performed. SPT was considered positive when the wheal diameter was ≥ 3 mm over the negative control. Subsequently an ICT was performed with 1:100, 1:10, and undiluted rhC1INH. ICT was considered positive when the mean erythema diameter was equal to or exceeding the positive control. Lastly, patients underwent a SC challenge with 0.14 mL, 1.4, 4.2, and 8.2 mL rhC1INH (corresponding to 21, 210, 630, and 1230 U rhC1INH, respectively), in total corresponding to the volume of one vial of 14 mL (2100 U) rhC1INH. This SC testing was performed under intense observation and monitoring, in a setting where all precautions were taken and equipment was readily available to treat hypersensitivity reactions, including anaphylaxis. The SC challenge was interpreted as a positive immediate type hypersensitivity reaction if one or more of the typical manifestations occurred: generalized itching, urticaria, angioedema, gastro-intestinal symptoms, dyspnea, wheezing, hypotension.[6] Local swelling and erythema were measured after predefined observation times, and reported as adverse event (AE) prior to dose escalation.

In vitro testing

In all patients, serum IgE to rabbit dander, cow's milk extract, alpha lactalbumin, beta lactoglobulin, and casein was measured by ImmunoCAP to confirm sensitization (Thermo Fisher Scientific, Uppsala, Sweden).

A direct basophil activation test (BAT) was performed to evaluate the relative contribution of possible allergens, if any, in case an immediate type hypersensitivity reaction to rhC1INH would occur. In the current study several allergens were tested including cow's milk, rabbit dander, rhC1INH and individual allergens from cow's milk and rabbit milk.

Basophil activation was analyzed using a flow cytometry-based assay measuring CD63 expression. Heparinized whole blood was stimulated at 37°C for 30 minutes with allergens diluted in RPMI/IL-3 (2 ng/ml, R&D systems, Minneapolis, MN, USA). Cow's milk and rabbit allergens were diluted to the following concentrations: 0.001, 0.01, 0.1, 1, 10, and 100 µg/ml. rhC1INH was used in the following concentrations: 0.00125, 0.0125, 0.125, 1.25, 12.5, and 25 mg/ml. The reaction was stopped by adding 25 µl of cold PBS/EDTA (20 mM). Cells were stained using PE-conjugated mouse-anti-human CD63, FITC-conjugated mouse-anti-human CD123 (both BioLegend, San Diego, CA, USA), and APC-conjugated mouse-anti-human CD203c (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Red cells were lysed using FACS Lysing Solution (BD Biosciences, San Jose, CA, USA). Basophil activation, expressed as percentage of CD63-positive cells within CD203c/CD123-positive cells, was analyzed by flow cytometry using a FACS Canto II (BD Biosciences).

Safety

Since AEs and serious adverse events (SAEs) before any exposure to rhC1INH cannot be related to rhC1INH, only treatment-emergent AEs (TEAEs) and treatment-emergent serious adverse events (TESAEs) according to Good Clinical Practice guideline SAE definitions are reported.[7] Safety was evaluated by recording TEAEs and TESAEs, their time of occurrence after exposure to rhC1INH and their intensity and causality to rhC1INH. Causality was reported by the investigators on a 5-point scale, including not related, remote/unlikely, possible, probably or definitely related to the study medication. Subsequently, TEAEs graded as possible, probably or definitely related were considered to be "related" for purposes of reporting. Only TEAEs reported to occur within the first 24 hours after any exposure to rhC1INH could be considered related.

During the testing, local allergic reactions could be treated symptomatically with oral antihistamines, and systemic hypersensitivity reactions could be treated with intravenous antihistamines, steroids and/or intramuscular epinephrine.

Analysis

In estimating the sample size for determining the negative predictive value (NPV) of the combination of SPT and ICT for the outcome of the SC challenge, we assumed that at least one patient would have an immediate type hypersensitivity reaction during SC challenge. To obtain a NPV with a 95% confidence interval, in order for the lower limit of the confidence interval to be at least 80%, data needed to be available for 25 evaluable subjects with negative SPT and ICT to rhC1INH.

The study was to be stopped if more than two patients with negative SPT and ICT developed systemic symptoms of hypersensitivity upon subcutaneous challenge with rhC1INH, or if more than 10 patients had a positive skin test (SPT or ICT).

Statistical analysis was performed using SAS software, Version 9.2 for Windows. It consisted of descriptive statistics for the quantitative variables including IgE results and skin test results, and frequency distributions for the ordinal and nominal variables including demographic, and allergy data. For the serological specific IgE (sIgE) tests regarding RA and CMA as mentioned above, values below or above the limit of detection are reported as 0.00 or >100, respectively, and all sIgE data are described using the median and quartiles.

The NPV of the combination of the SPT and ICT for the outcome of the SC challenge was defined as the ratio of patients without systemic symptoms of hypersensitivity following SC challenge with rhC1INH, over the number of patients having tested negative for both the SPT and ICT.

RESULTS

Study participants and allergy characteristics

In total 26 patients, 15 with RA, 6 with CMA, and 5 with both RA and CMA (Online Repository Table E1) were included in the study (Figure 1). Eleven others were not eligible to participate since RA and CMA could not be confirmed by SPT or IgE. Mean age was 33.7 years (SD 11.4), 17 (65.4%) were female. All 26 included patients were highly atopic and suffered from at least one atopic disease in addition to their RA and/or CMA, a majority of 24 (92.3%) had allergic rhinitis, 18 (69.2%) asthma, and 18 (69.2%) atopic eczema (Table E1).

Symptoms to rabbit exposure consisted of rhinitis, conjunctivitis, sneezing, feeling of swelling in the throat, and in some cases wheezing or dyspnea. CMA patients had symptoms that varied from oral allergy symptoms to anaphylactic shock shortly after ingestion of cow's milk. In 3 patients CMA was confirmed by double-blind placebo controlled food challenge (DBPCFC) as part of standard care. Median sIgE titer to rabbit dander in patients with a positive SPT to rabbit dander was 3.2 kU/L (range 0.16 - 26.3). The median sIgE titer to cow's milk extract in those with a positive SPT to cow's milk was 13.2 kU/L (range 0.32 - >100) (Table E1). All

included patients had a positive SPT to rabbit dander and/or cow's milk: 16 were positive to rabbit only, 5 to cow's milk only, and 5 to both. In subjects with only positive SPT to rabbit dander, the median wheal diameter was 6.0 mm (range 3 to 12) and the median difference with the negative control was 5.5 (range 3 to 12). In subjects with only positive SPT to cow's milk (n=5) median wheal diameter was 7.0 mm (range 5 to 15) and the median difference with the negative control was 5.5 mm (range 3 to 12), respectively.

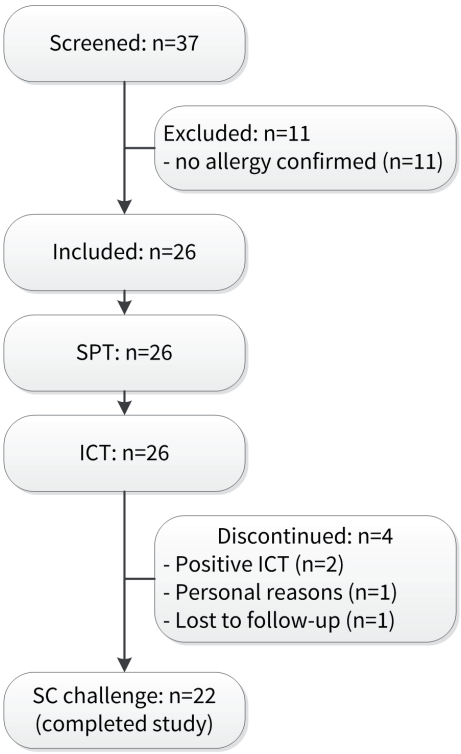

Figure 1:

Flowchart of in– and exclusion of patients

SPT: skin prick test, ICT: intracutaneous test, SC challenge: subcutaneous challenge

Results of skin prick test and intracutaneous test with rhC1INH

Skin test results are shown in table 1. None (95% CI: 0.0% ; 13.2%) of the subjects had a positive SPT with 1:10 diluted, and undiluted rhC1INH according to the defined criteria. Therefore, all 26 study participants proceeded to ICT. In the 1:100 and 1:10 dilutions of rhC1INH none had erythema exceeding the positive control. Two of the 26 patients had a positive ICT to undiluted rhC1INH (7.7%, 95% CI: 0.9; 25.1) but not to the diluted drug. The two patients had erythema on ICT greater than positive control by 1 mm and 4 mm. They did not experience any immediate type hypersensitivity symptoms as described in the methods section. Both patients were allergic to rabbit dander but not to cow's milk.

Results of subcutaneous challenge with rhC1INH

The two patients with a positive ICT did not proceed to the SC challenge with rhC1INH. Two others were lost to follow-up or discontinued study participation due to personal reasons (figure 1). The remaining 22 patients underwent SC challenge. Although patients could experience local symptoms, further described in the safety section below, none experienced symptoms suggestive for an immediate type hypersensitivity reaction. The NPV of the combination of SPT and ICT was defined as the ratio of patients without systemic symptoms of hypersensitivity following SC challenge with rhC1INH, over the number of patients having

Table 1: Results of skin tests with rhC1INH

Patient	Diagnosis	SPT wheal diameter (mm)				ICT erythema diameter (mm)					SC challenge
		Negative control	1:10 rhC1INH	Undiluted rhC1INH	SPT rhC1INH	Positive control	1:100 rhC1INH	1:10 rhC1INH	Undiluted rhC1INH	ICT rhC1INH	Type I hypersensitivity reaction
1	RA	0	0	0	neg	29	10	8	8	neg	no
2	RA	0	0	0	neg	24	23	15	15	neg	no
7	RA	0	0	0	neg	24	9	4	0	neg	no
10	RA	0	0	0	neg	32	8	7	0	neg	lost to FU
11	RA	0	0	0	neg	32	18	18	19	neg	no
17	RA	0	0	0	neg	35	10	13	11	neg	lost to FU
18	RA	0	0	0	neg	42	33	23	16	neg	no
21	RA	0	0	0	neg	33	11	7	14	neg	no
25	RA	0	0	0	neg	42	24	19	29	neg	no
28	RA	0	0	0	neg	29	12	8	17	neg	no
30	RA	0	0	0	neg	36	13	16	37	pos	n.d.
31	RA	0	0	0	neg	23	8	8	11	neg	no
34	RA	2	0	0	neg	29	9	16	18	neg	no
37	RA	0	0	0	neg	39	11	17	17	neg	no
38	RA	0	0	0	neg	27	21	20	31	pos	n.d.
4	CMA	2	0	0	neg	37	10	10	0	neg	no
6	CMA	0	0	2	neg	36	2	6	7	neg	no
8	CMA	0	0	0	neg	38	6	8	16	neg	no
12	CMA	0	2.8	0	neg	21	7	6	10	neg	no
13	CMA	0	0	0	neg	35	0	0	28	neg	no
19	CMA	0	0	0	neg	36	0	10	0	neg	no
5	CMA+RA	0	0	0	neg	32	5	22	20	neg	no
14	CMA+RA	0	0	0	neg	28	9	0	0	neg	no
15	CMA+RA	0	0	0	neg	28	0	0	10	neg	no
23	CMA+RA	0	0	0	neg	25	6	6	0	neg	no
32	CMA+RA	0	0	0	neg	36	27	16	20	neg	no

SPT: skin prick test, ICT: intracutaneous test, SC: subcutaneous, neg: negative, pos: positive, FU: follow-up. N.d.: not done.
SPT positive when wheal diameter ≥ 3 mm over the negative control. ICT positive when mean erythema diameter equal to or exceeding the positive control.

tested negative for both the SPT and ICT. Therefore the NPV of the combination of SPT and ICT for the outcome of the SC challenge was 100% (95% CI: 84.6% ; 100%).

In vitro testing

A BAT was performed in all 26 included patients (Figure 2). When using the clinical definitions of RA and CMA as described above (history and sensitization by SPT and/or IgE) we could classify 18/26 patients (69%) correctly. One of the RA patients without CMA showed basophil activation after stimulation with cow's milk extract, and vice-versa two CMA patients without RA responded after stimulation with rabbit extract. One of the RA and CMA patients responded only after stimulation with rabbit extract. Thirteen patients (52%) did not reach

a plateau in the percentages of CD63-positive cells. Three patients had a negative BAT, and one additional patient showed spontaneous basophil activation resulting in uninterpretable results for these patients. In none of the patients, basophil activation was observed upon stimulation with rhC1INH.

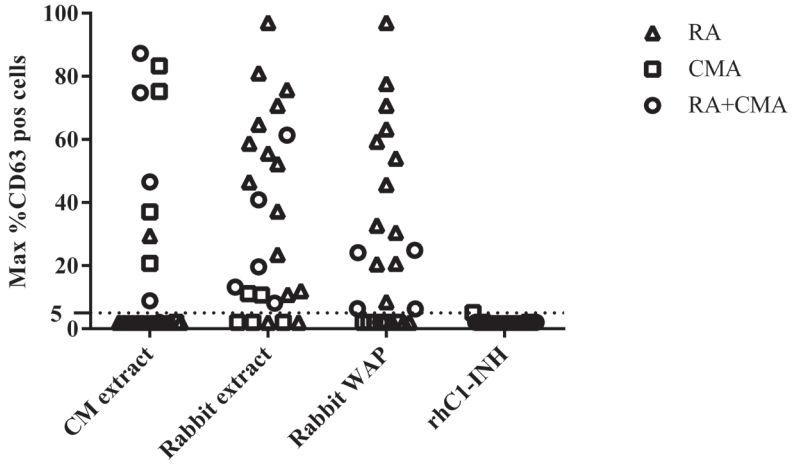

Figure 2: Basophil Activation Test results

RA: rabbit allergy, CMA; cow's milk allergy. BAT results are positive for each patient when the percentage of CD63-positive cells exceeds 5% (dotted line)

Safety

Local TEAEs within 24 hours after exposure to rhC1INH were observed in 7 patients (32%) after SC challenge (Table 2). In 4 (18%) of them this was considered possibly, probably or definitely related to the study medication. Local TEAEs were considered mild in all patients. Furthermore, 4 patients reported general TEAEs after SC challenge of which 1 case of headache of moderate severity was drug related (4.5%), which is a listed adverse drug reaction of rhC1INH.[8] Local AEs after ICT included painful injection in 15 patients, as is commonly reported for ICTs in daily practice.[6] No TESAEs were reported during the study.

DISCUSSION

This is the first study that investigates the allergenic potential of rhC1INH in patients diagnosed with an allergy to rabbit dander and/or cow's milk. None of the 22 patients, who had a with negative SPT and ICT to rhC1INH, showed as an immediate type hypersensitivity reaction during the drug challenge with rhC1INH. The NPV of the combination of SPT and ICT for the outcome of the SC challenge was 100% (95% CI: 84.6% ; 100%).

Table 2: Treatment–emergent adverse events ≤ 24 hours after exposure to rhC1INH

	TEAE		Related TEAE	
	n	%	n	%
Total number of patients reporting ≥1 TEAE	19	73.1	14	53.8
Total number of patients reporting ≥1 administration-related (local) TEAE	17	65.4	14	53.8
SPT	0	0	0	0
ICT	15	57.7	12*	46.2
SCC	7	31.8	4	18.2
Total number of patients reporting ≥1 other (general) TEAE	9	34.6	2	7.7
SPT	0	0	0	0
ICT	6	27.3	1	3.8
SCC	4	18.2	1*	3.8

TEAE: Treatment-emergent adverse event. ICT: intracutaneous test. SCC: subcutaneous challenge. Local TEAEs included pain, swelling, feeling of tight skin, and hematoma. General TEAEs included abdominal pain, (feeling to) faint, worsening of eczema, rhinorrhea and eye pain. Related general TEAEs consisted of nausea after ICT, and headache after SCC. One patient reported headache and nausea. *Moderate severity in 1 patient (mild in the others).

The originally calculated required sample size of 25 patients anticipated that one may have a reaction to the SC challenge. Since this did not occur in any of the included patients, the NPV of the combination of SPT and ICT for the outcome of the SC challenge could be calculated after study completion of 22 patients, without impairing the lower value of the 95%-confidence interval (84.6% ; 100%). A limitation of the study is that rhC1INH was not injected intravenously (i.v) as it is approved for use. For safety reasons we used the subcutaneous administration route as is accepted in diagnostic drug challenges,[9] and refrained from i.v. administration. SPT and ICT with immediate readings are commonly used for investigation of immediate hypersensitivity reactions in drug allergy.[10] Plasma-derived C1INH has been administered i.v. for several decades, and recent studies applying the SC route of administration for these preparations indicate that similar safety results can be expected for these two routes of administration.[11]

In a previous study in 130 HAE patients and 14 healthy controls, five subjects had preexisting anti-rabbit epithelium IgE antibodies; one of them was a healthy subject with an undisclosed rabbit allergy, who developed an allergic reaction upon i.v. infusion with rhC1INH. The other four subjects with pre-existing sIgE levels to rabbit epithelium did not have an allergic reaction.[3] Since only one of those sensitized developed an allergic reaction, it is questionable whether a test for IgE against rabbit dander provides useful information to predict the risk for allergic reactions to rhC1INH.

Of the 22 patients who underwent a SC challenge with rhC1INH, 15 had RA. Their characteristics were comparable to a population described by Liccardi et al.[12] with similar clinical features including age, gender, and symptoms including rhinitis, conjunctivitis, and asthma symptoms, whereas median sIgE titers in that study were somewhat lower: 1.4 kU/L (range < 0.35 to > 100) versus 3.2 kU/L (range 0.16 to 26.3) in the current study. This suggests that our RA population was representative. Our CMA group (11 patients) covered the whole range of mild to severe cow's milk allergic patients, including two patients with a history of anaphylactic shock. Five patients (19%) even suffered both RA and CMA. Taken together, our study population represents the spectrum of RA and CMA patients with emphasis on the severe end of the spectrum.

None of the patients had a positive SPT to rhC1INH. Two RA patients showed a positive ICT (erythema larger than positive control) to undiluted rhC1INH only. Therefore they did not undergo the SC challenge with rhC1INH according to the study protocol. However, the reactions were considered irritative and not allergic, since the ICT was negative in diluted concentrations. Test substances in high concentrations can cause reactions even in healthy individuals. It is essential that non-irritant test concentrations are used and many drugs or drug classes are therefore diluted 1:10 or more for ICT to avoid irritant test concentrations.[9,10] For rhC1INH the threshold of irritant test concentrations for intradermal testing was not established, since this would require a drug and/or formulation only challenge in skin test positive subjects. This was not the aim of this study and not intended in the study protocol. The assumption of a nonspecific false positive skin test reaction to undiluted rhC1INH is also supported by the fact that unspecific erythema due to ICT was seen in all study subjects and in all diluted test concentrations. In all patients, including patients with a skin reaction only marginally smaller than the positive control, subcutaneous challenges did not result in allergic symptoms to rhC1INH. Furthermore, *in vitro* testing by BAT was negative for rhC1INH in all patients including both ICT positive patients. One of the patients with positive ICT had the highest IgE level for rabbit extract measured in this study. The healthy study subject with allergic symptoms after injection with rhC1INH as mentioned previously, also had a high sIgE level.[3] These numbers, however, are negligible to draw conclusions on this topic.

None of the 22 patients with negative SPT and ICT had an allergic reaction during the SC challenge with rhC1INH, confirming that administration in patients allergic to CM, rabbit, and even to both CM and rabbit, can be considered safe for the large majority of patients. This is supported by the fact that the clinical characteristics and sensitisation profile are representative for the RA and CMA population, probably even in cases with severe allergy. This is further supported by our BAT results. Considering that whey proteins are present in relatively high concentrations in milk of all mammalian species, the basophils of RA and CMA patients (or both) responded, as expected, to rabbit whey proteinsbut not to rhC1INH.[13,14] During clinical development and to date since market approval in 2010, there have been

no clinical reports of allergic events to rhC1INH in CMA patients. Additionally, in previous safety studies regarding rhC1INH, three subjects with sIgE to cow's milk allergens did not show allergic symptoms after exposure to rhC1INH.[3] Although no evidence from daily practice points toward safety concerns in CMA patients, and no evidence for cross reactivity between rabbit milk allergy and cow's milk allergy has been reported, the current study was not powered for a subgroup analysis.

Analysis of adverse events showed that performing SPT and ICT with rhC1INH, as well as subcutaneous administration of rhC1INH, was safe. Only a minority of patients reported adverse events, mostly mild and local, including 3 patients who reported pain during subcutaneous administration of rhC1INH. AEs regarding local swelling, and a local feeling of tightness of the skin, were related to the injected volume. The safety of subcutaneous administration of plasma-derived C1INH was shown very recently in case reports[15,16] and in a phase II trial.[11] All studies reported similar TEAEs related to the route of drug administration, but in higher percentages.[17,18] Amongst the other TEAEs related to rhC1INH only known side effects were reported in the current study.

In conclusion, none of the patients with negative SPT and ICT for rhC1INH had allergic symptoms during rhC1INH challenge. The negative predictive value of the combination of SPT and ICT for the outcome of the SC challenge was 100% (95% CI: 84.6% ; 100%). SC administration of rhC1INH was well-tolerated.

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Table E1: Individual demographic and allergy data

			History		SPT		Specific IgE						Other atopic diseases		
Patient	Age (y)	Gender	Rabbit	Cow's milk	Rabbit	Cow's milk	Rabbit extract	Cow's milk extract	Alfa-Lac (bos d 4)	Beta-Lac (bos d 5)	Caseine (bos d 8)	Diagnosis	Allergic rhinitis	Asthma	Atopic eczema
1	49	f	+	-	+	-	5.0	0.33	0.02	0.07	0.45	RA	+	-	+
2	26	f	+	-	+	-	2.33	0.12	0.01	0.08	0.03	RA	+	+	+
7	37	m	+	-	+	-	2.59	0.04	0.02	0.03	0.05	RA	+	-	+
10	39	m	+	-	+	-	5.1	0.12	0.02	0.03	0.05	RA	+	-	+
11	20	f	+	-	+	-	1.50	0.11	0.04	0.03	0.04	RA	+	+	+
17	23	f	+	-	+	-	4.4	0.04	0.02	0.01	0.02	RA	+	+	+
18	45	f	+	-	+	-	0.19	0.02	0.01	0.02	0.01	RA	+	+	-
21	44	f	+	-	+	-	4.2	0.11	0.03	0.06	0.05	RA	+	+	+
25	31	f	+	-	+	-	0.16	0.04	0.02	0.04	0.03	RA	+	-	+
28	19	f	+	-	+	-	1.32	0.06	0.07	0.08	0.09	RA	+	-	-
30	30	f	+	-	+	-	3.8	0.11	0.01	0.11	0.03	RA	+	+	+
31	24	f	+	-	+	-	6.5	0.36	0.25	0.42	0.35	RA	+	+	+
34	25	f	+	-	+	-	1.51	0.02	0.01	0.02	0.01	RA	+	+	+
37	43	f	+	-	+	-	0.54	0.05	0.02	0.03	0.03	RA	+	-	-
38	46	m	+	-	+	-	26.3	0.06	0.03	0.04	0.02	RA	+	+	+
4	54	f	-	+	-	+	0.00	25.0	2.31	9.27	22.8	CMA	+	+	-
6	33	m	-	+	-	+	0.02	13.2	1.20	0.51	5.4	CMA	-	-	-
8	20	m	-	+	-	+	0.09	> 100	5.2	8.5	> 100	CMA	-	+	-
12	49	m	-	+	+	+	2.97	28.6	9.9	5.0	43.0	CMA	+	+	+
13	34	f	-	+	-	+	0.36	0.32	0.03	0.46	0.11	CMA	+	-	+
19	22	m	-	+	-	+	0.05	1.01	0.03	1.62	0.21	CMA	+	+	+
5	22	m	+	+	+	+	0.48	6.84	1.67	1.30	8.42	CMA+RA	+	+	-
14	24	m	+	+	+	+	12.7	59	10.9	1.33	70	CMA+RA	+	+	+
15	47	f	+	+	+	+	0.50	10.6	0.02	0.55	14.0	CMA+RA	+	+	+
23	23	f	+	+	+	-	4.2	0.36	0.33	0.15	0.11	CMA+RA*	+	+	-
32	48	f	+	+	+	+	8.6	67	39	29.7	43	CMA+RA	+	+	+

+: positive, -: negative, SPT: skin prick test, Alfa-Lac: alfa-lactalbumin, Beta-lac: beta-lactoglobulin, RA: rabbit allergy, CMA: cow's milk allergy, na: not applicable. For patient 12 the SPT was positive for rabbit dander but the patient is not allergic according to the medical history, and for patient 23 the SPT was negative for cow's milk but the subject is allergic according to the medical history and IgE value.

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PART II:

TREATMENT OF URTICARIA WITH OR WITHOUT ANGIOEDEMA

CHAPTER 5:

EFFECTIVENESS AND SAFETY OF ANTIHISTAMINES UP TO FOURFOLD OR HIGHER IN TREATMENT OF CHRONIC SPONTANEOUS URTICARIA

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ABSTRACT

Background:
Treatment with second-generation antihistamines is recommended in patients with chronic spontaneous urticaria (CSU). Some patients remain unresponsive even after up-dosing up to fourfold. Many third line treatment options have limited availability and/or give rise to significant side effects. We investigated effectiveness and safety of antihistamine treatment with dosages up to fourfold and higher.

Methods:
This retrospective analysis of patients' records was performed in adult CSU patients suffering wheals and/or angioedema (AE). Demographic, clinical, and therapeutic data was extracted from their medical records. We recorded the type, maximum prescribed dosage, effectiveness, and reported side effects of antihistamine treatment.

Results:
Of 200 screened patients, 178 were included. Treatment was commenced with a once daily dose of antihistamines. Persisting symptoms meant that up-dosing up to fourfold occurred in 138 (78%) of patients, yielding sufficient response in 41 (23%). Up-dosing antihistamines was necessary in 110 (81%) patients with wheals and 28 (64%) with AE only (p=0.039). Of the remaining 97 patients with insufficient response, 59 were treated with dosages higher than fourfold (median dosage 8, range 5-12). This was sufficient in 29 patients (49%). Side effects were reported in 36 patients (20%), whereof 30 (17%) experienced somnolence. Side effects after up-dosing higher than fourfold were reported in six out of 59 patients (10%).

Conclusion:
Up-dosing antihistamines higher than fourfold dosage seems a feasible therapeutic option with regards to effectiveness and safety. The need for third line therapies could be decreased by 49%, with a very limited increase of reported side effects.

INTRODUCTION

Chronic urticaria is either inducible (CINDU) or spontaneous (CSU) or both [1, 2]. Angioedema (AE) can occur concurrently with urticaria in up to 40% of cases, and may occur alone in up to 10–20% of cases [3]. Patients suffering CSU can have wheals only, AE only, or both [1].

The therapeutic approach of chronic urticaria aims at symptom relief. Licensed doses (1 tablet daily) of modern second-generation antihistamines (sgAH) are the first line treatment. An increase in the dose only up to fourfold is recommended as second line treatment [1, 4]. However, every third to fourth patient will remain symptomatic despite up-dosing up to fourfold [5], hence alternative treatments are needed for (partially) unresponsive patients [1]. Current third-line – in the US guideline fourth-line – treatment options consist of omalizumab, cyclosporine A (CsA) or leukotriene receptor antagonist montelukast [1, 4]. However, each of these options has limitations: omalizumab is expensive and not reimbursed worldwide. CsA has a high incidence of adverse effects. For leukotriene receptor antagonists, the level of evidence for efficacy is low [1].

In our tertiary center, refractory patients were treated with antihistamines at varying dosages (including dosages higher than fourfold), in order to avoid the use of CsA as omalizumab had not yet been approved for treatment of CSU. Despite a lack of controlled studies, experts have reported benefit of dosing antihistamines higher than fourfold in CSU patients [6]. The objective of this study was to investigate the frequency of ineffectiveness of treatment with antihistamines up to fourfold the standard dose in patients with CSU, and to determine the effectiveness and safety of antihistamine treatment above fourfold the standard dose.

METHODS

Study design and subjects

A retrospective analysis of patients' records was performed in patients visiting our tertiary dermatology and allergology clinic for the evaluation of chronic urticaria and/or angioedema in 2012 (before registration of omalizumab), and for each patient all available data were collected up to 2014. Adult patients suffering CSU (wheals and/or AE for at least 6 weeks) were selected. All patients with other diagnoses including acute urticaria (duration of symptoms less than 6 weeks), CINDU including symptomatic dermographism, urticaria or angioedema caused by allergy or of other known causes, urticaria pigmentosa and urticaria vasculitis were excluded. Medical records were screened to verify inclusion and exclusion criteria. To be recognized as a representative sample, 159 patients were needed (based on a margin of error of 5%, a confidence interval of 95% and an eligible population of 268 patients) [7]. To have a representation of both AE patients and patients with wheals, all 100 available AE patients and 100 additional patients with wheals were screened for inclusion in the study. Patients with wheals were randomly selected based on their unique patient identification number in the electronic medical record system; dossiers of the patients with the lowest numbers were

screened until 100 patients with wheals were included.

Data were collected as described below, and used in strictly anonymous form, according to the code of conduct for medical research approved by the hospital's Medical Ethical Committee. Written informed consent for the publication of this report was not required from the patients, as approved by the Ethics Committee, protocol number 13-459.

Treatment regimen

The local treatment protocol, as shown in figure 1, commenced with the approved dosage of antihistamine, and in case of persisting symptoms up-dosing occurred up to fourfold. Higher than fourfold dosages were only used in patients who remained symptomatic at fourfold antihistamine dosages. Treatment adjustments were performed individually by all prescribing physicians of the department. Patients often were already on antihistamine treatment prior to their first visit at the clinic. In this case, they did not have to start at the licensed dosage, but could further follow the local protocol. All treatment was open. At the start of the study, standard disease-specific questionnaires were not yet available and therefore not used.

Data collection

After inclusion, data was collected from electronic patient records. Data regarding demographic and therapeutic characteristics until 2014 was extracted manually from the electronic medical records from each patient's first visit to the

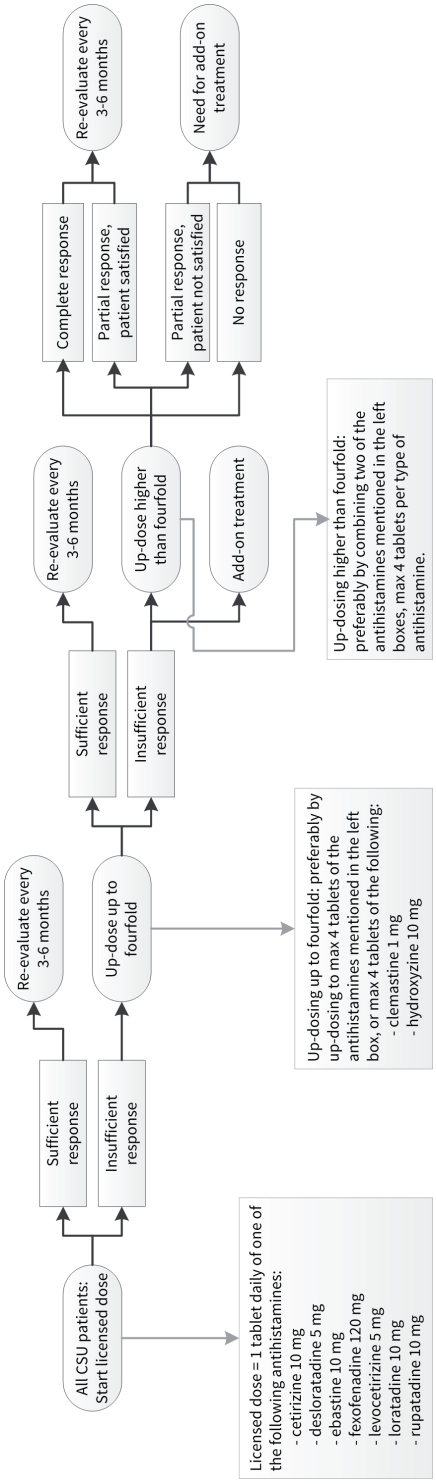


Figure 1: Local treatment protocol
When patients were already on antihistamine treatment prior to their first visit at the clinic, they could further follow the local protocol. Evaluation was planned every 3-6 months, or earlier if symptoms are intolerable.

clinic. Outcome variables were the type of antihistamines patients were treated with, the maximal prescribed dosage, treatment results, and reported side effects.

For each patient antihistamine use was recorded as daily treatment as well as rescue medication., The type, maximal prescribed dosage, treatment results including clinical symptoms of wheals, angioedema, and itch, and reported side effects were also recorded. Antihistamines were prescribed prior to or during consultations at this tertiary hospital. In the Netherlands, all types and dosages of antihistamines are reimbursed, hence prescribing is not affected by insurance. The doctor's reported effect from each treatment option was allocated by the investigators into one of two categories: sufficient or insufficient. Disagreements were discussed and resolved. When the dose of antihistamines was raised and further information was missing, it was interpreted that lower doses did not reach sufficient response. The reported effect from dosages higher than fourfold was further subdivided into four different categories: 1) no effect 2) insufficient effect and patient not satisfied, 3) partial disease control, and patient satisfied, or 4) completely free of symptoms. If information was unclear category allocation was performed by two investigators. Up-dosing higher than fourfold was preferably performed by combining more than one type of antihistamine. In these cases the effect of one specific antihistamine was unclear and was not included for analysis. In case of side effects, the type of side effect as reported in the medical record, as well as the corresponding eliciting dosage of antihistamines, were recorded. Additional blood tests were not performed routinely.

Analyses

Descriptive statistics were performed using IBM SPSS Statistics version 21. To explore differences in the proportion of patients with sufficient or insufficient effect from antihistamines in the three subgroups of patients (wheals only, AE only, and both wheals and AE), patients with unknown effect of treatment were excluded, and the Pearson Chi-Square (chi-square) test was used. The Fisher-Freeman-Halton exact (Fischer's exact) test was used in cases of low numbers.

RESULTS

Population

Of the 200 screened patients, 178 patients (121 [68%] female; median age 48.2 years [range 20-87]) were diagnosed with CSU and were included in the study, including 10 patients who suffered both CSU and CINDU. Five of 200 were excluded due to angioedema with known causes (1 with HAE, 1 with specific allergy, and 3 with ACEi-AE) and 17 were excluded since they had only inducible symptoms (CINDU). Of the included 178, 43 patients (24%) had wheals only, 44 (25%) had AE only, and the remaining 91 (51%) suffered both symptoms. The median disease duration before the first consultation at our University referral center was 1 year (range 0 to 41.5 years). Ninety-four patients (53%) reported that they had previously

visited another dermatologist or allergologist for evaluation of wheals and/or AE. All visits per patient were reviewed, and this comprised a median number of visits of 2 (range 1-57) and an additional median number of 2 consultations per telephone (range 0-24).

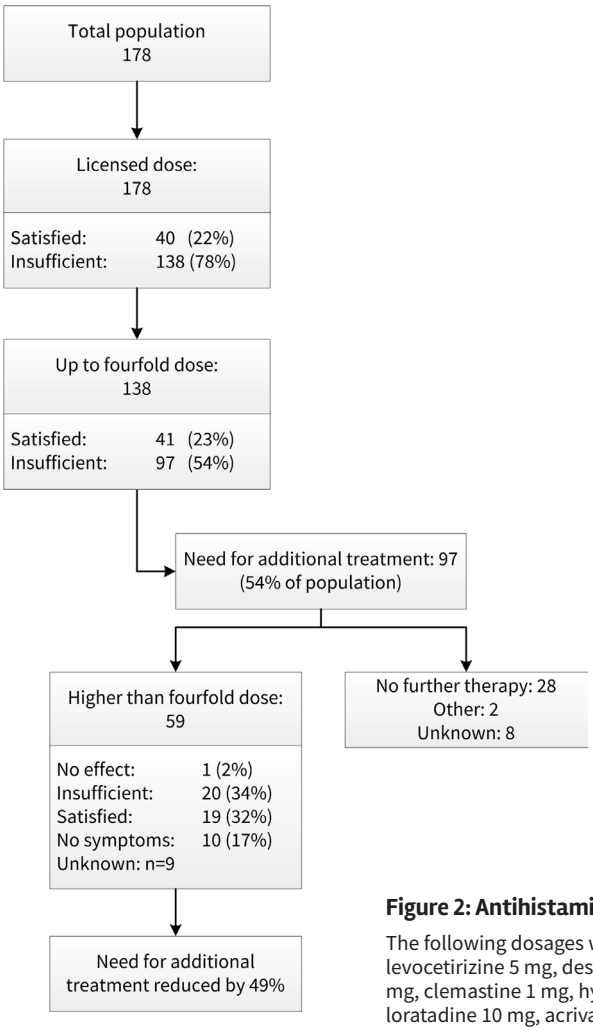


Figure 2: Antihistamine dosages and results

The following dosages were considered as standard dose: levocetirizine 5 mg, desloratadine 5 mg, fexofenadine 180 mg, clemastine 1 mg, hydroxyzine 25 mg, cetirizine 10 mg, loratadine 10 mg, acrivastine 8 mg three times daily.

Maximum doses and effectiveness of antihistamines

All 178 included patients were initially treated with the licensed, once daily, dosage of antihistamines (Figure 2). Of them, 27 patients (15%) used antihistamines only on demand. In 138 patients (78%) the licensed dose was ineffective and in all these refractory patients the dose was raised up to fourfold. This remained ineffective in 97 (70%). Subsequently, 59 of these 97 patients were treated with higher doses of antihistamines by combining 2 types of second generation antihistamines with a maximum of eightfold the licensed dose. The median maximal combined dose of antihistamines in these 59 was eightfold (range 5 –

8), however in 8 individuals the dose was raised further (range 9 - 12). Ten of 59 patients (17%) subsequently became completely free of symptoms, and nineteen patients (32%) had sufficient results. Thus, in 49% of patients a higher than fourfold dose reduced or completely eliminated symptoms. The remaining 38 of the 97 refractory patients received no further treatment (n=28), or further treatment was unknown (n=8), or they received other types of therapy including ultraviolet (UV) treatment (n=2), for which effectiveness results were not included in the current study (Figure 2).

Need for up–dosing in wheals versus AE without wheals

Up-dosing up to fourfold was necessary more frequently in patients with wheals (wheals only: 35 of 43 patients; 81%, wheals and AE: 75 of 91 patients; 82%) than in AE without wheals (28 of 44 patients; 64%; p=0.039). However, when looking specifically at those with only one of the two symptoms, there was no statistically significant difference between wheals only and AE only (p=0.053, Table 1a).

A trend was observed that up-dosing higher than fourfold was also necessary more often in patients with wheals (17; 40%) compared to AE only (7; 16%; p=0.056, Table 1a).

Response to antihistamine dosages higher than fourfold dichotomized as sufficient (29 patients [58%]) versus insufficient (21 patients [42%]) did not differ between the three diagnosis groups (p=0.530, Table 1b), or between patients with wheals only or AE only (p=0.620).

Types of antihistamines

The 178 patients received a total of 354 antihistamine prescriptions. As shown in Table 2, the most frequently prescribed antihistamines were levocetirizine (71% of patients), desloratadine (56%) and fexofenadine (23%). A total of 35 patients (20%) were treated with clemastine, and 26 patients (15%) were treated with hydroxyzine. Since 12 patients were treated with both hydroxyzine and clemastine at any time during their disease, a total of 49 patients (28%) received first-generation antihistamines (fgAH). All patients who were treated with fgAH were refractory to licensed doses: 13 (27%) received fgAH as part of up-dosing up to fourfold, and the remaining 36 (73%) received fgAH in addition to sgAH to reach total dosages of antihistamines higher than fourfold. Clemastine was up-dosed in 11 patients up to 3 mg per 24 hour period, and hydroxyzine in nine patients up to 75 mg per 24 hours.

Safety of antihistamines

Of the 178 patients 36 (20%) reported side effects upon treatment with antihistamines independent of the dosage. Fifteen of 36 patients reported side effects for two (n=14) or three (n=1) different antihistamines (Table 3). Somnolence (Figure 3a) was reported in 30 of 36 patients (83%), including 5 patients (10%) treated with fgAH and 28 (16%) with sgAH. Six out

Table 1: Table 1a shows the frequencies of up–dosing. Table 1b shows the effectiveness of antihistamine dosages higher than fourfold.

1a: frequencies of up–dosing

Symptoms	Licensed dose n (%)	Up to fourfold n (%)	Higher than fourfold n (%)	Total n (%)
AE only	16 (36)	21 (48)	7 (16)	44 (100%)
Wheals only	8 (19)	18 (42)	17 (40)	43 (100%)
AE and wheals	16 (18)	40 (44)	35 (38)	91 (100%)

Percentages are shown per row to enable comparison between diagnoses groups. Patients are shown in their maximum dosage group, thus patients who received fivefold or higher have previously been treated with lower doses. Numbers therefore differ from Figure 1. There was no statistically significant difference in frequency of up-dosing between the three groups (chi-square p=0.053), and also not between those with wheals only (included for analysis: n=35) and AE only (n=28; chi-square p=0.056). N.a.: not applicable.

1b: effectiveness of antihistamine dosages higher than fourfold.

Symptoms	Insufficient* n (%)	Sufficient n (%)	No symptoms n (%)	Total n (%)
AE only	**2(33)**	**2 (33)**	**2 (33)**	**6 (100%)**
Wheals only	7(58)	3 (25)	2 (17)	12 (100%)
AE and wheals	12(38)	14 (44)	6 (19)	32 (100%)

Percentages are shown per row to enable comparison between diagnoses groups. Effect of treatment was unknown in 9 patients, the numbers of patients therefore differ from table 1a.
*One patient suffering wheals only reported no effect of up-dosing to fivefold or higher, this case is included in the group of patients with insufficient effect. There was no statistically significant difference in treatment result between the three groups (Fischer's exact p=0.530) nor in those with wheals only (included for analysis: n=17) and AE only (n=7, Fischer's exact p=0.620).

of 36 (17%) reported side effects only during treatment with dosages higher than fourfold (Figure 3b). They consisted of somnolence in 5 patients and were unclear in 1. Vomiting or diarrhea were not reported by any of the patients.

DISCUSSION

In CSU patients refractory to up to fourfold doses of antihistamines, higher than fourfold dosages reduced or completely eliminated symptoms in an additional 49%. Side effects were reported in 20% of patients and consisted mainly of somnolence. After up-dosing higher than fourfold only 6 out of 59 patients (10%) reported side effects.

In more than half of the total population response remained insufficient despite antihistamine treatment up to fourfold, consistent with previous studies [8–10]. Up-dosing higher than fourfold, with a median dose of 8 tablets daily, was effective in half of patients including those

Table 2: Frequency of use and frequency of satisfying result per antihistamine

Antihistamine	Frequency n (%)	Sufficient effect of licensed dose n (%)	Sufficient effect after up-dosing n (%)	Dose with sufficient effect median (range)
Levocetirizine 5 mg	126 (71)	15 (12)	26 (21)	2 (0-6)
Desloratadine 5 mg*	99 (56)	1 (1)	15 (15)	4 (1-6)
Fexofenadine 180 mg	41 (23)	5 (12)	2 (5)	1 (0-2)
Clemastine 1 mg	35 (20)	1 (3)	2 (6)	2 (1-2)
Hydroxyzine 25 mg	26 (15)	0 (0)	1 (4)	3 (n.a.)
Cetirizine 10 mg*	16 (9)	2 (13)	2 (13)	1.5 (1-4)
Loratadine 10 mg	9 (5)	0 (0)	1 (11)	2 (n.a.)
Acrivastine 3x8 mg	2 (1)	0 (0)	0 (0)	n.a.

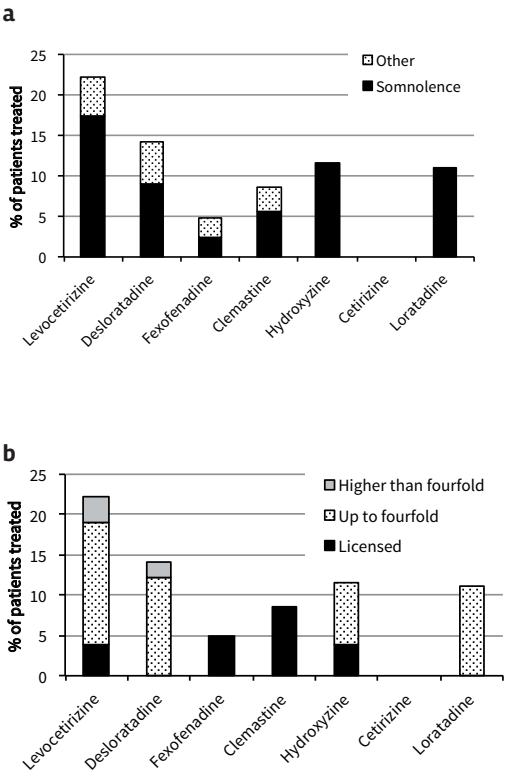
Frequency data are presented as numbers and percentages of the total population (n=178) , and frequencies of sufficient response are presented as percentages of those treated with the specific antihistamine.*in 1 patient it was unknown which dose caused sufficient effect. N.a.: not applicable. Please note that in most patients where up-dosing higher than fourfold occurred, this was done by combining more than one type of antihistamine. In these cases the effect of one specific antihistamine was unclear and was not included in this analysis.

Table 3: Frequency of side effects per antihistamine

Antihistamine	Frequency n (%)	Somnolence n (%)	Other n (%)	Other side effects
Levocetirizine 5 mg	28 (22)	22 (17)	6 (5)	Weight gain (n=2), palpitations, increase of symptoms, unclear (n=2)
Desloratadine 5 mg	14 (14)	9 (9)	5 (5)	Palpitations, headache, increase of symptoms (n=2), unclear (n=2)
Fexofenadine 180 mg	2 (5)	1 (2)	1 (2)	Increase of symptoms
Clemastine 1 mg	3 (9)	2 (6)	1 (3)	Increased intra-ocular pressure
Hydroxyzine 25 mg	3 (12)	3 (12)	0 (0)	n.a.
Cetirizine 10 mg	0 (0)	0 (0)	0 (0)	n.a.
Loratadine 10 mg	1 (11)	1 (11)	0 (0)	n.a.
Acrivastine 3x8 mg	0 (0)	n.a	n.a	n.a.

Data are presented as numbers and percentages of patients treated with this antihistamine. Patients may have reported side effects upon treatment with more than one antihistamine, therefore the numbers do not match the total number of patients reporting at least one side effect. “Other” side effects occurred in one patient each, unless otherwise specified. Percentages are rounded and may therefore not match within one row. Please note that a low frequency of side effects may be due to a low frequency of use for the specific antihistamine, and to a lack of updating in the study population since only patient-reported side effects were shown.

Figure 3: Frequency of side effects, by a) type of side effect, and b) maximum dose



The following dosages were considered as standard dose: levocetirizine 5 mg, desloratadine 5 mg, fexofenadine 180 mg, clemastin 1 mg, hydroxyzine 25 mg, cetirizine 10 mg, loratadine 10 mg, acrivastine 8 mg three times daily.

with wheals only, with AE only, and with both symptoms. This is promising, since antihistamines have low costs as opposed to CsA or omalizumab, and they are available worldwide [1]. There is a lack of rationale for the dosage of fourfold being the maximum. In contrast, in both CSU and in cold urticaria, treatment with 4 tablets per day was shown to be more effective than 3 tablets per day, which in turn was more beneficial than 2 tablets or 1 tablet per day, indicating that higher doses could be more effective [9, 11]. Moreover, hydroxyzine is prescribed up to 200 mg/day, and because 30 mg of hydroxyzine equals about 10 mg cetirizine [11]. Two-hundred mg equals a dose of 60 mg cetirizine/day, considerably higher than fourfold. Since such high dosages of hydroxyzine are used in daily practice, it was likely that higher dosages of other antihistamines could also be effective. Additionally, hydroxyzine is a first generation antihistamine with considerably more side-effects than cetirizine. We conclude that many patients indeed had a favorable response to higher doses of antihistamines when doses up to fourfold were insufficient.

The effect of antihistamines, but only up to fourfold, has been studied previously, but very few head-to-head studies have been performed [9, 12]. Some studies have examined antihistamines up to fourfold [9, 13], or four tablets daily [8]. The latter may be somewhat confusing, for instance for fexofenadine where both 120 mg and 180 mg tablets are available. There are also studies available where only twofold dosages were the maximum [10]. Some studies showed preponderance of efficacy of higher dosages in the treatment of chronic spontaneous urticaria [8–10, 14], and cold and cholinergic urticaria [15-18]. In contrast, in some other studies comparable efficacy of standard and higher dosages was found [19–22].

The most frequently used antihistamines were sgAH. The use of fgAH is discouraged in the European guideline [1] since serious side-effects of these old sedating antihistamines have been reported, including lethal overdoses. Additionally, in the elderly they increase the risk of impaired cognition, inattention, disorganized speech, altered consciousness, and falls [1]. Yet, a substantial number of patients was treated with fgAH at some time during their disease: clemastine was prescribed to 20% and hydroxyzine to 15%. It was previously suggested that some physicians were not fully aware of the content of the most recent guidelines and therefore did not follow them [23]. However, the successful use of fgAH after failure of treatment with sgAH has been described [24]. Furthermore, the US guideline does support the use of fgAH in patients who do not achieve control of their condition with higher-dose second-generation antihistamines [4]. Our results support that the addition of not only sgAH but also of fgAH can lead to sufficient disease control when either licensed doses of sgAH, or dosages up to fourfold had failed.

Somnolence was reported by a minority of patients. It is well known that somnolence is one of the most reported unwanted effects of antihistamines. It occurs even when using sgAH [9] in up to 23% of patients [8], and it does not significantly increase when comparing with baseline somnolence [9], or when antihistamine doses are increased [8]. This was confirmed in our study for even higher dosages. Patients treated with fgAH did not report sedation more often than those treated with only sgAH. A possible explanation for this is that fgAH were mostly used in low dosages in addition to high dosages of sgAH, whereas often times relatively high doses of fgAH are used [24–26]. Very few of the side effects (10%) were reported only when antihistamine dosages were raised higher than fourfold. For desloratadine it was previously shown that dosages up to 9-fold did not lead to clinically relevant adverse effects [27]. The low frequency of somnolence in the current study is likely to be an underestimation of unwanted effects due to missing information or recall bias, and since patients were not all actively asked about side effects, including but not limited to somnolence. It could also be caused by tolerance to somnolence which can develop within 4 days of subsequent use of H1 antihistamines [24, 28]. It was hypothesized that this is caused by adapted neuropharmacological effects [28]. On the other hand, it remains difficult to distinguish somnolence caused by treatment from somnolence caused by sleep disturbances due to the disease [8, 9]. Pruritus is most bothersome during the evening and at night when it makes falling asleep difficult and wakes patients later in the night. This causes chronic fatigue with a direct impact on QoL and physical and emotional well-being [5]. Still, although the influence of prolonged treatment on somnolence may be limited, and improvement of urticarial symptoms reduces somnolence [9], urticaria patients report sleep difficulties almost twice as often as control subjects [29], and our results support that somnolence occurred in a minority of urticaria patients [8].

A limitation of this study is the retrospective design. Therefore, precise documentation of results of treatment was missing in some patients. Also, there was a lack of objective measurements of effectiveness. With regard to side effects, we presented these as collected from the medical records. Somnolence was the most frequently named side effect. Liver and kidney function tests were not performed routinely. However, the extent of missing information was rather limited and different results are therefore not expected. Furthermore, the EAACI/GA2LEN/EDF/WAO urticaria guideline does not recommend to combine antihistamines [1], since the mechanism of action of sgAH is similar and mixing different antihistamines would therefore theoretically not have additional benefits [12]. In the current study we combined different antihistamines. This was performed in case dosages higher than fourfold were given, to limit side-effects related to a specific antihistamine. Lastly, CSU is a self-limiting disease. In the current study spontaneous remission may have occurred and this would then be misinterpreted as effectiveness of treatment.

In conclusion, we show that by up-dosing antihistamines higher than fourfold, half of patients reached sufficient treatment response while causing a limited increase in side effects. The need for other third line therapies could be decreased considerably. These findings need to be confirmed in a prospective controlled study. The results are of special interest in case of side effects or contraindications to currently proposed third line treatments, or when they are locally not (yet) available.

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CHAPTER 6:

OMALIZUMAB IN PATIENTS WITH CHRONIC SPONTANEOUS URTICARIA: A SYSTEMATIC REVIEW AND GRADE ASSESSMENT

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SUMMARY

Chronic spontaneous urticaria (CSU) is characterized by the occurrence of hives, angioedema or both for a period of at least 6 weeks. Many patients remain symptomatic despite treatment with H1 antihistamines, even at higher doses. This systematic review assessed the quality of the evidence for the effects of omalizumab as treatment in patients with CSU. We searched PubMed, the Cochrane Database of Systematic Reviews and the Cochrane Central Register of Controlled Trials up to 7 August 2014. Three review authors independently carried out study selection, risk of bias assessment and data extraction. Two review authors analysed the data. Five randomized controlled trials (RCTs), which included 1116 participants, were evaluated. All the RCTs were judged as having a low risk of bias. There was a statistically significant improvement in measures of disease activity and quality of life following treatment with omalizumab when compared with placebo [mean difference (MD) −11.58, 95% confidence interval (CI) −13.39 to −9.77 and MD −13.12, 95% CI −16.30 to −9.95, respectively]. Complete response and partial response were more frequent after treatment with omalizumab [risk ratio (RR) 6.44, 95% CI 3.95–10.49 and RR 4.08, 95% CI 2.98–5.60, respectively]. There was no difference in the proportion of participants reporting adverse events between the omalizumab and placebo treatment groups (RR 1.05, 95% CI 0.96–1.16). There was high-quality evidence to support the effectiveness and safety of omalizumab 300 mg per month for the treatment of CSU for up to 6 months.

INTRODUCTION

Chronic spontaneous urticaria (CSU) is characterized by recurrent itchy weals (hives), angioedema or both, that occur for at least 6 weeks and have no external trigger.[1] Similar symptoms may also be induced by a demonstrable stimulus including, but not limited to, cold, heat, vibration or exercise, which is classified as chronic inducible urticaria. CSU, formerly known as chronic idiopathic urticaria, may lead to a severe impairment in the quality of life of an individual.[2] One in five people will experience at least one episode of urticaria during their lifetime, with a point prevalence of up to 0.6%. Women are affected nearly twice as often as men with a peak incidence of between 20 and 40 years of age. Although CSU can resolve within months to a year, a considerable number of people suffer for more than 5 years (10–50%).[3]

Mast cells, basophils and immunoglobulin (ig) E have been implicated in the pathophysiology of chronic urticaria.[4] A recently updated guideline (EAACI/GA2LEN/EDF/WAO) recommended modern second-generation antihistamines followed by an increase in dosage if symptoms persist, as the first two steps in the treatment algorithm for CSU.[1] Treatment options for patients where there is a lack of response to H1antihistamines include ciclosporin, leukotriene-receptor antagonists and short term use of systemic corticosteroids. However, many patients remain symptomatic or suffer from side-effects, more especially with ciclosporin treatment. Omalizumab, a recombinant humanized monoclonal antibody, which selectively binds to human IgE, was initially licensed for the treatment of allergic asthma, and has recently received approval from the European Medicines Agency and the U.S. Food and Drug Administration (FDA) for the treatment of CSU.[5] Omalizumab may have a beneficial role in the treatment of CSU by reducing mast cell and basophil activation mediated by IgE and its high-affinity receptor (FcεRI) on the surface of target cells, thereby reducing the levels of free IgE and the FcεRI-receptor.[6, 7]

The EAACI/GA2LEN/EDF/WAO guideline on urticaria recommends omalizumab as add-on therapy to modern second-generation H1 antihistamines as third line in the treatment algorithm of urticaria. This guideline was revised and updated using a 'modified version of GRADE'. However, for the corresponding Dutch guideline, which is currently under preparation, we have more comprehensively followed the GRADE [8] approach to assess the quality of the evidence for the effectiveness and safety of omalizumab. Our results are summarized in this systematic review.

MATERIALS AND METHODS

We conducted a systematic review of randomized controlled trials (RCTs) evaluating the evidence for the effectiveness and safety of omalizumab for chronic spontaneous urticaria.

Search strategies

We searched PubMed, the Cochrane Database of Systematic Reviews and the Cochrane Central Register of Controlled Trials up to 7 August 2014 (Table 1). International guidelines were examined for further potentially eligible RCTs.[1, 9] Two review authors (M.C.U. and M.T.v.d.E.) assessed the titles and abstracts identified from the searches and independently evaluated each study to determine whether predefined selection criteria were met.

Table 1. Search strategy (search date: 7 August 2014)

PubMed. Search date: 07-08-2014	The Cochrane Database of Systematic Reviews and the Cochrane Central Register of Controlled Trials Search date: 07-08-2014
1. chronic urticaria[tiab]	1. Chronic urticaria:ti,ab
2. chronic urticaria	2. Chronic urticaria
3. "Urticaria"[Mesh]	3. "Urticaria"
4. chronic spontaneous urticaria[tiab]	4. chronic spontaneous urticaria:ti,ab
5. chronic idiopathic urticaria[tiab]	5. chronic idiopathic urticaria:ti,ab
6. #1 OR #2 OR #3 OR #4 OR #5	6. omalizumab:ti,ab
7. omalizumab[tiab]	7. omalizumab
8. "omalizumab" [Supplementary Concept]	8. anti-immunoglobin E Therapy:ti,ab
9. anti-immunoglobulin E therapy[tiab]	9. anti-IgE:ti,ab
10. anti-IgE[tiab]	10. #1 or #2 or #3 or #4 or #5
11. #7 OR #8 OR #9 OR #10	11. #6 or #7 or #8 or #9
12. #6 AND #11	12. #10 and #11
Limit: Abstract available	Results 20 (15 trials / 5 reviews)
Results: 160	

Inclusion criteria

Included were RCTs assessing the efficacy and safety of omalizumab in patients with CSU (Table 2). Other study designs and those investigating participants with inducible urticaria were excluded.

Outcome measures

We rated the clinical importance of each outcome on a nine-point scale according to the recommendations in the GRADE Handbook.[8] In total we considered seven prespecified outcomes. The first three were considered to be critical outcomes for decision-making: (i) mean change in disease activity from baseline; (ii) mean change in quality of life from baseline; (iii) proportion of patients with adverse events. The next three were considered to be important outcomes for decision-making (iv) proportion of patients with a complete response; (v) proportion of patients with a partial response; (vi) proportion of angioedema-free days. The last outcome was considered of limited importance: (vii) proportion of participants that experienced remission within 1 month.

Table 2. Characteristics of included studies

Study	Methods	Participants	Interventions	Outcomes
Kaplan et al. 2013 GLACIAL	Phase III RCT double blind, placebo-controlled, multicentre trial. Study performed in Australia, Germany, New Zealand, Poland, United Kingdom, the united States, Singapore.	335 patients with chronic spontaneous urticaria despite treatment with H1-antihistamines, plus H2-antihistamines, LTRAs of both. Omalizumab n=252, Placebo n=83 - CIU/CSU for 6 months or longer; - Age: Range 12-75 years (Germany > 18 years) - UAS7 ≥16 - ISS≥8	24 weeks. Follow-up 16 weeks. - 6 subcutaneous injections at 4-week intervals of either 300mg omalizumab or placebo. - Rescue medication (diphenhydramine 25mg, up to a maximum of 3 doses per 24-hour period) was allowed. - Throughout the treatment period, participants were required to maintain stable doses of their prerandomisation combination therapy with H1-antihistamines plus H2-antihistamines, LTRAs of both.	Primary endpoints: - Adverse events at week 24 - Severe adverse events at week 24 Secondary endpoints: - Change from baseline in mean weekly ISS at week 12. - Change from baseline in mean the UAS7 at week 12. - Proportions of patients with a UAS7 <6 at week 12. - Time to achieve a minimal important difference (MID) in weekly ISS at week 12. - Proportion of patients who were hive and itch free (UAS7=0) at week 12. - Proportion of angioedema free days from week 4 to 12 - Change in health-related quality of life, as measured by using DLQI. Exploratory endpoints: - Change in health-related quality of life, as measured by using and CU-Q2oL at week 12. Exploratory endpoints were also evaluated at week 24.
Saini et al. 2014 ASTERIA I	Phase III randomised, double-blind, placebo-controlled, multicentre trial. Study performed in the United States, Denmark, France, Germany, Poland, Italy, Spain en Turkey.	319 patients with chronic spontaneous urticaria who remained symptomatic despite treatment with approved doses of H1-antihistamines. Omalizumab 75mg (n=78), Omalizumab 150mg (n=80) Omalizumab 300mg (n=81) Placebo (n=80) - CIU/CSU for 6 months or longer and itching for more than 8 consecutive weeks - Age: Range 12-75 years - UAS7 ≥16 - ISS≥8	24 weeks. Follow-up 16 weeks - 6 subcutaneous injections at 4-week intervals of either 75mg, 150mg, 300mg omalizumab or placebo. - Rescue medication (diphenhydramine 25mg, up to a maximum of 3 doses per 24-hour period) was allowed. - For the first 12 weeks, participants were required to maintain stable doses of their prerandomisation H1-antihistamine treatment. - During weeks 13 to 24 patients were allowed to add on additional H1-antihistamines.	- Primary endpoint was the change from baseline in weekly ISS at week 12. Secondary endpoints included: - Change from baseline in UAS7 - Proportion of patients with a complete response (UAS7=0) - Proportions of patients with a UAS7 <6 - Proportion of weekly ISS MID responders. - Proportion of angioedema free days during weeks 4 to 12. Exploratory endpoints included the assessment of efficacy endpoints at week 24 and change from baseline to week 12 in CU-Q2oL.

Table 2 (Continued)

Study	Methods	Participants	Interventions	Outcomes
Maurer et al. 2013 ASTERIA II	Phase III randomised, double-blind, placebo-controlled, multicentre trial. Study performed in the United States, Denmark, France, Germany, Poland, Italy, Spain en Turkey.	323 participants with CIU who remained symptomatic despite the use of approved doses of H1-antihistamines. Omalizumab 75mg (n=82), Omalizumab 150mg (n=82) Omalizumab 300mg (n=79) Placebo (n=79) - CIU for 6 months or longer and hive and itching for more than 8 consecutive weeks - Age: Range 12-75 years - UAS7 ≥16 - ISS≥8	12 weeks. Follow-up 16 weeks. - 3 subcutaneous injections at 4-week intervals of either 75mg, 150mg, 300mg omalizumab or placebo. - Rescue medication (diphenhydramine 25mg, up to a maximum of 3 doses per 24-hour period) was allowed. - Throughout the treatment period, participants were required to maintain stable doses of their prerandomisation H1-antihistamines.	- Primary endpoint was the change from baseline in weekly ISS at week 12. Secondary endpoints included: - Change from baseline in UAS7 - Proportion of patients with a complete response (UAS7=0) - Proportions of patients with a UAS7 <6 - Proportion of weekly ISS MID responders. - Proportion of angioedema free days during weeks 4 to 12. Exploratory endpoints included the assessment of efficacy endpoints at week 24 and change from baseline to week 12 in CU-Q2oL.
Maurer et al. 2011 EXQUISITE	Phase II randomised, double-blind, placebo-controlled, multicentre trial. Study performed in 16 centres in Germany.	49 patients with chronic spontaneous urticaria and IgE autoantibodies against TPO with persistent symptoms despite standard antihistamine therapy. - Age: > 18 years - Diagnosis of CU with persistent symptoms for more than 6 weeks despite receiving maximal in label antihistamine therapy - Total serum IgE level between 30IU/mL or greater and 700 IU/mL or less. - Specific serum IgE-anti TPO antibody level of 5.0 IU/mL or greater within the last 3 months before enrolment. - UAS7>10	24 weeks. - Omalizumab 75-375mg (n=27) versus placebo (n=22) every 2- 4 weeks for 24 weeks. - Rescue medication (loratadine 10mg/day or clemastine 1mg/day) was allowed.	Primary endpoint was the change from baseline in mean weekly UAS after 24 weeks of treatment Secondary efficacy endpoints included: - Daily scores for wheals, pruritus, erythema and angioedema; - Use of concomitant medication; - Change in health-related quality of life, as measured by using the DLQI, Skindex-29 and the CU-Q2oL.
Saini et al. 2011 MYSTIQUE	Phase II randomised, double-blind, placebo-controlled, multicentre trial. Study performed in the United States and Germany.	90 patients with CIU (pruritus and hives for> 3 days in a 7-day period for > 6 consecutive weeks) despite treatment with on approved dose of an H1-antihistamine. Omalizumab 75 mg (n = 23) Omalizumab 300 mg (n = 25) Omalizumab 600 mg (n = 21) Placebo (n = 21)- Age: Range 12-75 years - UAS7 ≥12	4 weeks. Follow-up 12 weeks. - A single subcutaneous injection of 75 mg, 300mg, 600mg omalizumab or placebo. - Rescue medication (diphenhydramine 25mg, up to a maximum of 3 doses per 24-hour period (In Germany max 50mg) was allowed.	Primary endpoint was the change from baseline in mean weekly UAS after 4 weeks. Secondary outcomes included the change in weekly pruritus score and weekly score for the number of hives from baseline to week 4 (severe) adverse events

Data extraction and synthesis

Three authors (M.C.U., M.T.v.d.E. and E.J.v.Z.) independently assessed the risk of bias in the included studies following the criteria described in Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions.[10]

The same three authors (M.C.U., M.T.v.d.E. and E.J.v.Z.) extracted data using a previously developed data extraction form. Two authors (M.C.U. and E.J.v.Z.) entered data into Review Manager 2011 (RevMan).[11] We contacted Novartis Pharma to retrieve missing trial details and data. All evaluations were compared and disagreements between authors were discussed and resolved.

Continuous outcomes were presented as mean differences (MD) and dichotomous outcomes as risk ratios (RR). All outcomes were reported with their associated 95% confidence intervals (CIs) and were analysed in RevMan according to a random effects model using the Inverse Variance method for continuous outcomes and Mantel-Haenszel test for dichotomous outcomes, unless stated otherwise.[10] Heterogeneity between studies was assessed using the I2 statistic, where I2 > 60% was considered moderate to substantial.
We entered data into meta-analyses from studies evaluating omalizumab 300 mg and also data from one study covering a dose range of 75–375 mg in which the doses were calculated based on body weight and total serum IgE levels, according to the optimised dosing strategies used in the treatment of allergic asthma.[12]

GRADE profiler (GRADEpro) was used to rate the quality of evidence and to create a 'Summary of findings' table (see Table 3).[8]

RESULTS

Our searches retrieved 180 references to studies (see Fig. 1).

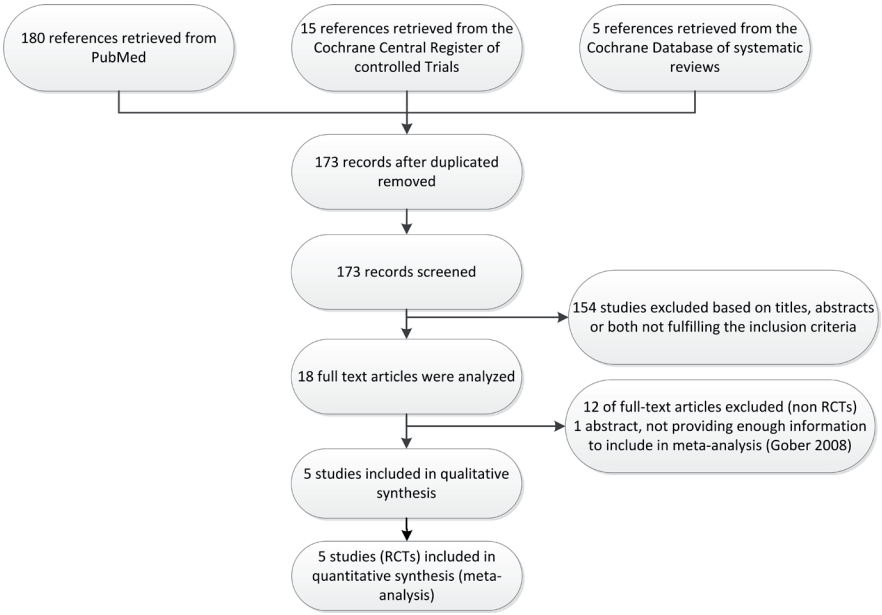
Description of included studies

Five RCTs, comprising 1116 participants, met our inclusion criteria (see Table 2).[13-17] Out of these, only the data for 749 participants receiving omalizumab 300 mg or 75–375 mg were entered into the meta-analyses. We identified one small study (20 participants) that was an abstract to conference proceedings, provided limited data, and therefore was not included in our review.[18]

Characteristics of the trial settings and methods

Three phase III studies (ASTERIA I, ASTERIA II and GLACIAL) and two phase II studies (MYSTIQUE and X-QUISITE) were included. All studies were double-blind, placebo controlled, multicentre trials, and had been conducted in recent years.

Figure 1. Study flow diagram.



Characteristics of the participants

The number of participants ranged between 49 and 335 per study. All had been diagnosed with CSU and only those with moderate to severe disease activity, despite receiving treatment, participated in the studies. In the X-QUISITE study only participants with CSU and IgE autoantibodies against thyroid peroxidase (TPO) were included.[13]

Characteristics of the interventions

Four RCTs evaluated omalizumab 300 mg,[14-17] three of which also evaluated other doses of omalizumab. One study evaluated omalizumab with a dose range of 75–375 mg vs. placebo.[13] Omalizumab was used as add-on treatment (mainly to H1-antihistamines) in three studies.[14, 15, 17] Rescue medication as either diphenhydramine, loratadine or clemastine was permitted in most studies.

Characteristics of the outcome measures

Patient-reported outcomes were assessed in daily diaries in all studies. The disease-specific Urticaria Activity Score (UAS) was used in all of the studies.[19] The UAS7, which is used to make daily assessments for 1 week, combines daily weal numbers and pruritus intensity and has a total score of 42 points. Most studies used the Itch Severity Scale (ISS), which forms part of the UAS7 and has a total score of 21 points. Our other critical outcome, quality of life, was assessed in four studies with the disease-specific Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL).[20] The CU-Q2oL consists of 23 items divided into six domains (pruritus, swelling, impact on life activities, sleep problems, limits and looks) and has a

Table 3. Summary of findings, in the setting of multicentre hospital trials, in relation to the question: Should omalizumab 300 mg be used in chronic spontaneous urticaria?

Quality assessment							No of patients		Effect	
No of participants (studies)	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Omalizumab 300mg**	**Control**	**Relative (95% CI)**	**Absolute**
Change in disease activity from baseline (treatment 4-24 weeks[4]; measured with: Change in UAS7 from baseline; range of scores: 0-42; Better indicated by lower values)										
749 (5 studies[5])	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	464	285	-	MD 11.58 lower (13.39 to 9.77 lower)
Change in quality of life from baseline (treatment 12-24 weeks[4]; measured with: Change in CU-Q2oL from baseline; range of scores: 0-115; Better indicated by lower values)										
703 (4 studies[6])	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	439	264	-	MD 13.12 lower (16.3 to 9.95 lower)
Adverse events (treatment 4-24 weeks[4]; assessed with: Any adverse events during treatment)										
749 (5 studies[5])	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	342/464 (73.7%)	183/285 (64.22%)	RR 1.05 (0.96 to 1.16)	32 more per 1000 (from 26 fewer to 102 more)
								48%		24 more per 1000 (from 19 fewer to 77 more)
								86%		43 more per 1000 (from 34 fewer to 138 more)
Complete response (treatment 4-24 weeks4; assessed with: UAS7=0 or complete resolution of urticarial symptoms8)										
749 (5 studies[5])	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	177/464 (38.1%)	16/285 (5.6%)	RR 6.44 (3.95 to 10.49)	305 more per 1000 (from 166 more to 533 more)
								5%		272 more per 1000 (from 148 more to 475 more)

Table 3 (Continued)

Quality assessment							No of patients		Effect	
No of participants (studies)	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Omalizumab 300mg**	**Control**	**Relative (95% CI)**	**Absolute**
Partial response (treatment 4-24 weeks[4]; assessed with: UAS7<6 or proportion of patients with 75% improvement in UAS7)										
700 (4 studies[9])	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	241/437 (55.1%)	36/263 (13.7%)	RR 4.08 (2.98 to 5.6)	422 more per 1000 (from 271 more to 630 more)
								9%		277 more per 1000 (from 178 more to 414 more)
								20%		616 more per 1000 (from 396 more to 920 more)
Angioedema free days (treatment 12-24 weeks[7]; measured with: Proportion of angioedema free days; range of scores: 0-100; Better indicated by higher values)										
576 (3 studies[10])	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	372	204	-	MD 5.66 higher (2.55 to 8.76 higher)
Begin of remission within 1 month - not measured										
- (0 studies)	-	-	-	-	-	-	-	-	-	-

1 Study Maurer 2011 used an 'asthma scheme' of omalizumab 75-375mg
2 All patients were patients with CSU who remained symptomatic despite treatment,
3 Study Maurer 2011 comprised patients with CSU and IgE autoantibodies against TPO with persistent symptoms despite standard antihistamine therapy
4 Subcutaneous injections of omalizumab or placebo every 2-4 weeks
5 Studies Saini 2014; Saini 2011; Kaplan 2013; Maurer 2011; Maurer 2013
6 Studies Saini 2014; Maurer 2011; Maurer 2013; Kaplan 2013
7 Subcutaneous injections of omalizumab 300mg or placebo every 4 weeks
8 Study Maurer 2011 assessed complete resolution of urticarial symptoms using investigator global assessment of symptoms
9 Studies Saini 2014; Saini 2011; Maurer 2013; Kaplan 2013
10 Studies Kaplan 2013; Maurer 2013; Saini 2014

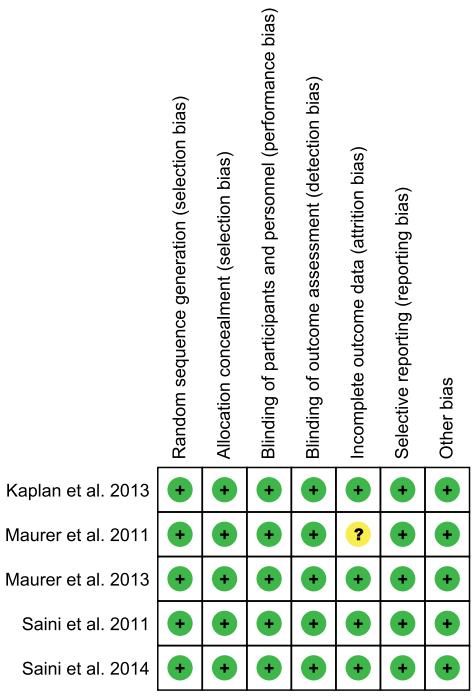
maximum score of 115 points. Four studies used generic questionnaires to assess quality of life, i.e. the Dermatology Quality Life Index (DLQI).[13-15, 17] The proportion of patients who reported adverse events was reported in all studies.

Complete response to treatment was assessed in all studies, with most using the UAS7 where a score of 0 indicated a complete response. One study assessed complete resolution of urticaria symptoms using an Investigator Global Assessment of symptoms. Three studies assessed partial response, which was represented by a UAS7 score of 6 or less.[14, 15, 17] One study quantified partial response as the proportion of patients with 75% improvement of symptoms compared with baseline.[16] Angioedema-free days were assessed in three studies while remission within 1 month was not addressed at all.

Risk of bias of included studies

We assessed the studies for risk of bias (see Fig. 2). All five studies were categorized as at 'low risk of bias', i.e. plausible bias unlikely to seriously alter the results. In all five studies the methods used to generate the allocation sequence were described in sufficient detail to enable a clear judgement. Sequence generation was carried out in four of the studies using an interactive voice and web response system. In the X-QUISITE study randomization was performed using a validated system. Central allocation was used in all studies. The methods used to achieve blinding of both participants and investigators were adequately described and reported. Blinding was achieved through the use of similar packaging and we were confident that adequate measures had been taken to blind participants and key study personnel. All prespecified outcomes mentioned in the methods section were fully addressed in the results. In all phase III studies, efficacy analyses were undertaken using data from a modified intention-to-treat population (randomized patients who received at least one dose of the study drug). Although in each of these three studies one participant was not entered into the analysis we judged this as a low risk of bias. The drop-out rates in the studies were moderate to low (10–16.9%). All dropout rates were balanced and therefore judged as low risk of bias, except

Figure 2. Risk of bias summary



for one study which we judged as unclear risk of bias.[3]. There was no baseline imbalance in urticaria severity between the study groups. As there was no selection, performance, detection or reporting bias, we did not consider industry sponsoring to pose an additional risk.

Effects of interventions

A summary of the key results is in Table 3. The quality of the body of evidence was rated high for all critical and important outcomes.

Mean change of disease activity from baseline

Pooled data from 749 participants demonstrated that the mean difference in UAS7 was 11.58 points lower after treatment with omalizumab compared with placebo (95% CI −13.39 to −9.77; $p < 0.001$). This improvement in measures of disease activity is clinically relevant because the minimal important difference (MID) of the UAS7 is estimated to be between 9.5 and 10.5 (see Fig. 3).[21]

Mean change of quality of life from baseline

Quality of life, assessed using the CU-Q2oL, improved by 13.12 on a scale of 0–115 points (95% CI −16.3 to 9.95; $p < 0.001$).[13-15, 17] The MID of the CU-Q2oL has not been established and therefore the clinical relevance of these data cannot be ascertained (see Fig. 4).

Proportion of patients with adverse events

Adverse events occurred in 73.7% (342 of 464) of the patients treated with omalizumab and in 64.2% (183 of 285) of the controls with no significant difference between the groups [risk ratio (RR) 1.05, 95% CI 0.96–1.16] (Fig. 5). Adverse events with an incidence of more than 1% and 2% higher than placebo during treatment in the phase III studies included headache, sinusitis, arthralgia and upper respiratory tract infections.[22] Injection-site reactions such as pain, swelling, erythema and pruritus were also reported (2.7% omalizumab 300 mg vs. 0.8% placebo).[22]

Proportion of patients with a complete response

Complete control of symptoms was achieved in 38.1% of the patients treated with omalizumab compared with 5.6% of the patients treated with placebo. This difference was statistically significant (RR 6.44, 95% CI 3.93–10.43; $p < 0.001$).

Proportion of patients with a partial response

Partial response was achieved in 55.1% in the active treatment group compared with 13.7% of the patients in the placebo group; this difference was statistically significant (RR 4.08, 95% CI 2.98–5.6; $p < 0.001$).[13-15, 17]

Figure 3. Improvement in disease activity (UAS7) from baseline

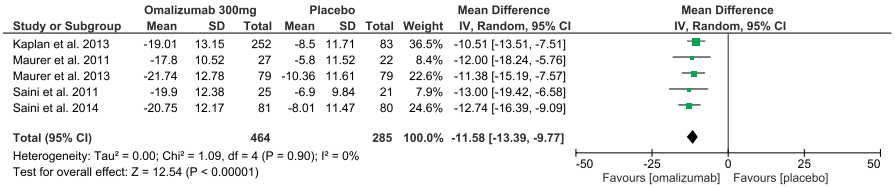


Figure 4. Improvement in quality of life (CU-Q2oL) from baseline

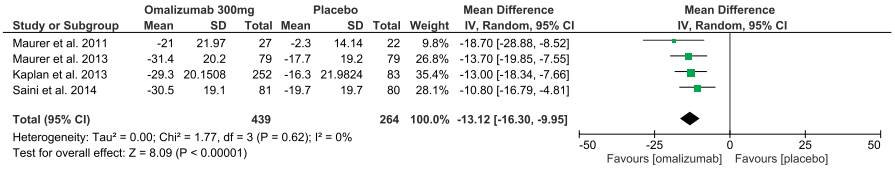
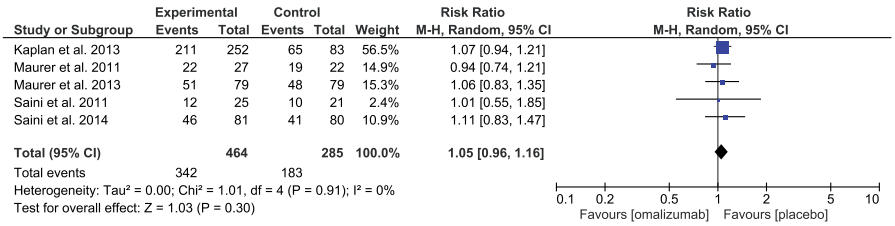


Figure 5. Proportion of participants with an adverse event



Proportion of angioedema–free days

The proportion of angioedema-free days (range: 0–100%) was assessed in 576 patients.[14, 15, 17] The mean proportion of angioedema-free days was 5.66% higher after treatment with omalizumab when compared with placebo (MD 5.66, 95% CI 2.55–8.76; p < 0.001). The proportion of angioedema-free days was also high in the placebo group (88.1–89.2%).

Proportion of participants that experienced remission within 1 month

This outcome was not assessed in any of the studies.

DISCUSSION

The overall quality of evidence as assessed using the GRADE approach for the effectiveness and safety of omalizumab for chronic urticaria was high. All of our critical outcomes were assessed in all the included studies with the exception of quality of life, which was reported in four out of the five studies.

In the GLACIAL study the primary objective was to assess the safety of omalizumab, whereas the other studies focused mainly on the effectiveness of omalizumab. Pooling of data was feasible for all of our outcomes, with the exception of the proportion of participants who achieved remission within 1 month, which was not assessed in any of the studies.

Three RCTs assessed the time to achieve the MID response (≥ 5 point decrease) in weekly itch severity score (range 0–21) at week 12. The median time to achieve this MID after 12 weeks of treatment ranged from 1.0 to 2.0 weeks.[14, 15, 17]

The EAACI/GA2LEN/EDF/WAO guideline on chronic urticaria provides a strong recommendation to use the validated UAS7 to assess disease severity.[1] The MID of the UAS7 is estimated at 9.5–10.5 points.[21] Pooled data from the five studies showed a statically significant and clinically relevant improvement in disease activity assessed using the UAS7.

Four studies emphasized the importance of quality of life as an outcome using the internationally accepted and validated CU-Q2oL instrument.[20] It is expected that the MID of this questionnaire will be established in the near future and will then provide more detail on the clinical relevance of this outcome.

Complete response and partial response was achieved more frequently after treatment with omalizumab than placebo, with a difference that was statistically significant. Furthermore, the proportion of angioedema-free days was higher after treatment with omalizumab compared with placebo, although the clinical relevance of this improvement (5.66%) remains unclear. However, it suggests that omalizumab may have an effect on angioedema.

A more pronounced reduction in use of rescue medication from baseline was observed in the omalizumab 300 mg group compared with the placebo group in four of the five studies. [13-15, 17] In one study a statistically significant reduction in number of diphenhydramine tablets per week was noted in favour of the omalizumab group [−4.2 (6.4) compared with −1 (5.2) in the placebo group; $p < 0.03$].[17] However, mean differences were not provided by the investigators in the other two studies[13, 15], and the mean difference was not statistically significant in Kaplan et al.[14]

There was no statistically significant difference in the proportion of participants experiencing adverse events between the groups. Although anaphylactic shock is a serious and well known adverse event associated with omalizumab in the treatment of allergic asthma, (estimated frequency of 1 in every 1000 injections), no cases of anaphylaxis attributed to omalizumab were reported.[22] It should be noted that the safety of omalizumab in the included studies has not been studied in patients undergoing treatment beyond 24 weeks. However, overall, omalizumab has a well-established good safety profile in allergic asthma.

Agreements and disagreements with other reviews

In the course of preparing the Dutch guideline on chronic urticaria, we carefully assessed the recently published EAACI/GA²LEN/EDF/WAO guideline.[1] This guideline used a 'modified version of GRADE' in translating the 'SIGN level of evidence into a GRADE quality of evidence', and the developers acknowledge the fact that additional quality criteria considered in GRADE were not assessed. In our review we provide a more detailed evaluation and reporting, which includes a full risk of bias assessment of individual studies as well as grading the quality of evidence for each outcome taking into account any limitations in study design or execution, inconsistencies in the results, indirectness of the evidence, imprecision and publication bias. Clinical decision-making on the choice of intervention for CSU should be based on high-quality evidence if available.

In the recently published systematic review of treatments for CSU with inadequate response to licensed first-line treatments, Mitchell et al. summarized evidence for treatments in patients that remain symptomatic despite treatment.[23] The scope of their review was broader, and included not only omalizumab, but leukotriene receptor antagonists and additional immunomodulating therapies, evaluated in a range of study designs. We used the Cochrane domain-based risk of bias tool for assessing the included studies, whereas Mitchell et al.[23] used a checklist recommended by the National Institute for Health and Clinical Excellence to assess methodological quality.[24] In addition, we contacted trial investigators to retrieve missing study details to enable more accurate judgements to be made regarding risk of bias assessments. Their search date was up to December 2011, and therefore did not include the three RCTs that were published after this date.[14, 15, 17] Although we are in concordance with the direction of their conclusions regarding the effects of omalizumab, we have provided a more detailed GRADE assessment to rate the quality of evidence in support of those conclusions.

A further systematic review which compared omalizumab with placebo for CSU identified the identical five studies.[25] Although they used the Cochrane risk of bias tool, they appear to have confused allocation concealment with blinding. They did not attempt to pool data into a meta-analysis and merely presented all data separately in tables. The reviewers concluded that the 300 mg dose was effective, but with more side-effects than with the lower doses. As with the other review no assessments of GRADE were undertaken.[20]

A recently published critical appraisal on the study of Maurer et al.[15] raised concerns about some methodological issues, the external validity and generalizability of the results.[26] There were additional concerns expressed about the trial protocol and history of changes, and in particular about discrepancies regarding prespecified and reported outcomes. The concerns were subsequently responded to satisfactorily by the investigators.[27]

The results of this review may not be generalizable to all participants with CSU in daily clinical practice for several reasons. Participants in the included studies had refractory disease, despite the use of previous medication (mostly H1 antihistamines). Only participants with moderate to severe disease activity were enrolled in the studies and thus one of the additional limitations was that the conclusions cannot be extrapolated to the most severe cases. Furthermore, participants under the age of 18 were excluded from all the studies.

Conclusion

There is high quality evidence for the effectiveness and safety of omalizumab 300 mg per month for the treatment of chronic spontaneous urticaria up to 6 months. Judgements about evidence and recommendations in healthcare are complex and GRADE assessments undertaken in this review can assist in clinical decision making and help formulate more accurate recommendations.

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CHAPTER 7:

MARKERS OF EFFICACY OF XOLAIR (OMALIZUMAB) IN CHRONIC SPONTANEOUS URTICARIA: PRELIMINARY RESULTS OF THE U-MEX STUDY

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Manuscript in preparation

ABSTRACT

Background

The exact pathogenesis of chronic spontaneous urticaria (CSU) and the mechanism of action of omalizumab in CSU remains unclear. Provided the essential role of the complement system in urticaria and the fast clinical response induced by omalizumab, we assessed the role of complement in adult patients with CSU prior to and during treatment with omalizumab.

Methods

In this monocenter prospective cohort study 18 CSU patients were treated with 6 administrations of 300 mg omalizumab. For analysis of complement components and additional inflammatory characteristics in the skin, two skin samples were taken per patient (baseline lesional and non-lesional), and blood samples at baseline, 1, 2, 6, and 24 hours and 1 and 2 weeks after the first omalizumab administration, and subsequently at every administration . Patient-reported outcomes assessed were: disease activity (urticaria activity score: UAS7), disease control (urticaria control test: UCT), and quality of life. Furthermore demographics, disease characteristics, medical history and concomitant treatment were carefully documented.

Results

Median age was 36.3 (range 21 - 68), 12 (67%) were female. Median UAS7 at baseline was 31.5 (interquartile range 25.8-38.0). After three and six months of treatment 8 patients (44%) and 9 patients (50%), respectively, reached a UAS7 ≤ 6 indicating well controlled disease. Lesional skin biopsies revealed edema in 9 patients, perivascular infiltration in 14 (78%), and interstitial infiltration in 7. Complement deposition (C4d) was seen at baseline in blood vessels in the papillary dermis of 11 patients (61%). In peripheral blood, C5a levels at baseline were elevated compared to healthy controls (p=0.0022). One hour after the first omalizumab administration a decrease in C3 (p=0.0002), C1q levels (p=0.0063), a trend for decrease in C3bc degradation products, C5a, and C5b-C9 membrane attack complex formation, and a trend for increase in C4bc complement degradation products was found, irrespective of disease activity. Five patients reported known adverse events related to omalizumab, no related SAEs nor cases of anaphylactic shock occurred.

Conclusion

Complement activation in CSU patients was illustrated in skin by C4d deposition, and in peripheral blood by elevated C5a levels. After treatment with omalizumab an immediate increase in complement activation products in peripheral blood was seen which was not related to clinical efficacy.

INTRODUCTION

Omalizumab is effective as third-line treatment in chronic spontaneous urticaria (CSU) patients, who fail to find relief with antihistamines up to fourfold.[1,2] It is a humanized monoclonal antibody which binds to free IgE, with a clinical response to occurring within the first 2 days of treatment in at least half of CSU patients.[3,4] Depletion of free IgE by omalizumab leads to a down-regulation of the FCεRI on mast cells and basophils,[5] by 11% after three days, by 78% after 10 days, and by 99% after 70 days in patients with allergic disease.[6] However, this down-regulation cannot explain the fast clinical response to omalizumab. Hence, there need to be other mechanisms contributing to the rapid clinical efficacy of omalizumab.

Sera from patients with urticaria can induce degranulation of basophils, and during this process the presence of intact complement is essential. In one study some patients IgG alone was able to degranulate basophils, whereas in other patients addition of complement lead to a marked increase in histamine release. In a second study more histamine release from basophils was seen upon increasing concentrations of C5a.[7–9] Additionally, the complement system is known for its rapid response upon activation. Furthermore, cutaneous mast cells express the complement C5a receptor whereas mucosal mast cells do not. This may explain why IgG anti-FcεRI autoantibodies in patients with urticaria in combination with complement would cause clinical symptoms which are limited to the skin.[10] It has been reported that C1q, C2, C3, C4, and C5 levels in peripheral blood are within normal limits in chronic urticaria.[11–13] No information has been published regarding complement degradation products in peripheral blood. Response of peripheral blood complement levels to treatment with omalizumab was also not studied before. Furthermore, it is unknown whether complement activation occurs in the skin. Complement activation in skin can be evaluated by determination of C4d deposition; a well-studied marker and a hallmark of complement activation, which is for instance also included in the BANFF criteria for humoral rejection after kidney transplantation.[14] Provided the essential role of the complement system in urticaria and the fast clinical response induced by omalizumab, efficacy of omalizumab in CSU may in part be caused by reduction of complement mediated inflammation.

The primary objective of this study was to determine whether the complement system was activated locally (in skin) and systemically (in peripheral blood) in adult patients with CSU treated with omalizumab. Secondary objectives were to a) determine the response of the complement system to omalizumab. and b) to relate the reduction of inflammatory characteristics in skin and peripheral blood to disease activity.

METHODS

Design and population

This monocenter exploratory prospective cohort study was performed in the University Medical Center Utrecht from 2015 until 2017. The current study is part of a broader study, and we now evaluate the first 18 patients who completed all study procedures.

Inclusion criteria were age 18 years or above, a diagnosis of CSU, and significant disease activity (weekly urticaria activity score (UAS7) ≥ 16 and in-clinic UAS ≥ 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)[15] and included a clearly defined underlying etiology for chronic urticaria (e.g. chronic inducible urticaria [CINDU]), a history of cancer, known hypersensitivity to omalizumab, and pregnancy. Routine administration of immunosuppressants including prednisolone and Cyclosporine A (CsA) 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. After a screening period of up to 2 weeks, eligible patients started treatment as described below, and a follow-up period of up to 2 months would follow. The latter could be shortened on patient's request if the UAS7 would 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).

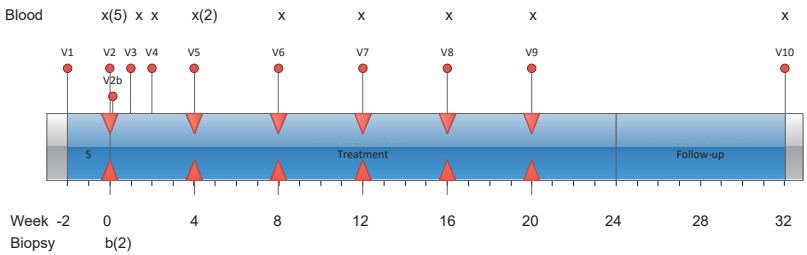
Omalizumab and concomitant medication

All patients received 6 doses of 300 mg omalizumab per 4 weeks (Figure 1). In addition, patients used H1 antihistamines up to fourfold throughout the study period, this was checked during every study visit. Leukotriene receptor antagonists (LTRA) or H2 blockers for indications other than CSU were permitted to continue during the study, otherwise it was attempted to discontinue those. 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. An observation time of at least 2 hours was maintained for the first three omalizumab administrations, and at least 30 minutes for further administrations.

Assessments in blood and skin

As shown in Figure 1, blood samples were collected at the following time-points: at baseline, after 1 hour, 2, 6, and 24 hours, after 1 and 2 weeks, and 4 weeks after the first administration of omalizumab. Subsequently, after the second dose, and prior to each subsequent dose blood was collected. 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 °C. Complement levels of C3 and C4 were determined

Figure 1: Study design per patient



S=Screening period, v=visit, x= venipuncture, b=biopsy. Triangles indicate that omalizumab is administered during the visit. Numbers in parentheses indicate the amount of time-points of the specific procedure to be performed during one visit. The duration of screening is flexible between week -2 and day 1, and the date of V10 is flexible between week 24 and 32.

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. Additionally, C1q in serum, and C3bc and C4bc in EDTA plasma were determined as previously described.[16]

Additionally, two 3 mm punch skin biopsies were taken at baseline: one from lesional and one from non-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[17,18] as 'not elevated' (0) or a mild, moderate or severe increase (1-3) compared to healthy skin, 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) as previously described, original magnification 400x.[18]

Clinical assessments

In female participants with childbearing potential, a urine pregnancy test was performed prior to the first administration of omalizumab. For safety evaluation, frequency and causality of all (serious) adverse events ([S]AEs) according to Good Clinical Practice guideline serious AE definitions were recorded.[19] Because (S)AEs before any exposure to omalizumab cannot be related to treatment, only (S)AEs after the first or subsequent administrations of omalizumab are reported.[19] Data regarding demographics, the presence of angioedema and/or CINDU in

addition to CSU, disease duration, medical history, and the use of concomitant treatment were collected. Several patient reported outcomes (PROs) were used: disease activity was measured throughout the study by using the UAS7.[20] Missing daily scores of the weekly disease activity scores after treatment was started were complemented by Last Observation Carried Forward method (LOCF). 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).[21] Quality of life (QoL) was measured at baseline, after 3 and 6 months of treatment, and at the last follow-up visit. For this purpose patients completed the Chronic Urticaria Quality-of-Life Questionnaire (CU-QoL),[22,23] and the Dermatology Life Quality Index (DLQI).[24]

Table 1: Patient and disease characteristics

Characteristic	Frequency or range
Median age in years (range)	36.3 (21 - 68)
Female, n (%)	12 (67)
Weight in median kg (range)	78.6 (61.4 – 108.6)
Body mass index median kg/m2 (range)	26.8 (21.1 – 44.1)
Presence of angioedema, n (%)	13 (72)
Presence of CINDU in addition to CSU, n (%)	6 (33)
Delayed pressure urticaria	4 (22)
Urticaria factitia	4 (22)
Family history of wheals or angioedema, n (%)	5 (28)
Median disease duration in years (range) , n (%)	2.1 (0.6 - 17)
Atopy by history, n (%)	
Any atopy	10 (56)
Atopic dermatitis	7 (39)
Asthma	3 (17)
Allergic rhinitis	4 (22)
Other allergy	6 (33)
Relevant comorbidities, n (%)	3 (17)
Depression	2* (11)
Previous episode of chronic urticaria	1 (6)
Medication use on day of OMA1, n (%)	
Second generation antihistamines (sgAH)	18 (100)
First generation antihistamines (fgAH)	2 (11)†
H2-antagonist	0
LTRA	1 (6)
H1-antihistamine dose on day of OMA1, n (%)	
Threefold	1 (6)
Fourfold	17 (94)
Previous switch of type of sgAH, n (%)	11 (61)
Previous use of systemic steroids, n (%)	8 (44)
Previous use of immunosuppressants, n (%)	5 (28)

Note that 2 patients with CINDU experienced more than one subtype and numbers therefore do not match within the table. The same accounts for comorbidies, as patients could have more than one comorbidity. *excluding one case of postnatal depression, †fgAH were used on demand in all patients. OMA1: day of first omalizumab administration.

Statistical analysis

Changes in inflammatory parameters in the skin will be related to changes in levels of circulating complement components, by using Spearman Rank correlation. Primary study parameters after treatment will be compared to baseline, and/or to the previous measurement, using Wilcoxon matched pairs signed rank tests, or paired samples T-test if appropriate. C3bc and C4bc activation ratios were determined by dividing the level of circulating C3bc or C4bc by the amount of C3 or C4 and multiplying the quotient by 100 to determine the percentage, as previously described.[16] For C5a, normal values were calculated based on results in a pool of 43 healthy volunteers (ethics committee protocol 07-125/C), and difference between volunteers and study population baseline scores was evaluated by Mann-Whitney U test. To test the hypothesis that complement levels change compared to baseline within one hour, a Bonferroni correction for multiple testing was applied. Since seven proteins were tested a p-value of 0.007 was considered statistically significant. To test the hypothesis that complement levels in peripheral blood are correlated to C4d deposition in skin, data were analyzed by McNemar test, and a similar correction was applied so a p-value of 0.007 was considered statistically significant. For other outcomes this significance level of 0.007 was also applied.

Descriptive analyses were carried out for all secondary outcomes. For PROs, a difference between each time-point and baseline was tested using Wilcoxon matched pairs signed rank tests, or paired samples T-test if appropriate. 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 will be 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, graphs were made using Microsoft Visio 2010 or GraphPad Prism version 6.02.

RESULTS

Population

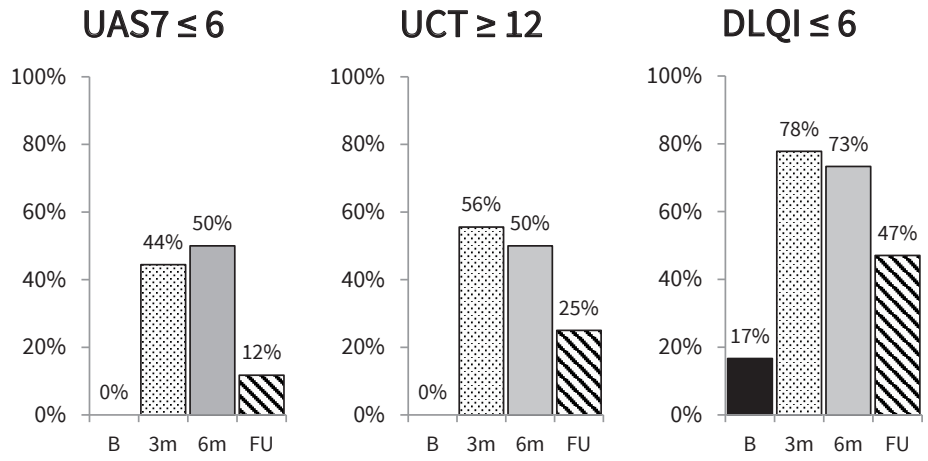
Of the 18 included patients, median age was 36.3 (range 21 - 68), 12 (67%) were female (Table 1). In addition to wheals, 13 (72%) reported angioedema. No patients reported hyper- or hypothyroidism, or a history of anaphylaxis.

Efficacy of omalizumab on disease activity, disease control and quality of life

Median PRO results throughout the study are shown in Table 2. As shown in Figure 2, after three months of treatment 8 patients (44%) reached well controlled disease activity defined as a UAS7 score of 6 or lower ($p < 0.001$). At the same time 10 patients (56%) had good disease control, defined as a UCT score of 12 or higher ($p < 0.001$), and 14 patients (78%) reported increase in QoL defined as a DLQI score of 6 or lower ($p < 0.001$). At the end of the treatment

period a higher proportion of patients reached these scores (UAS7:9 [50%], p < 0.001, UCT: 6 [50%], p=0.005, DLQI:11 [73%], p < 0.001.

Figure 2: Proportion of patients with UAS7 ≤ 6, UCT ≥ 12, and DLQI ≤ 6



UAS7: weekly Urticaria Activity Score, a score of 6 or lower indicates low or no disease activity. UCT: Urticaria Control Test, a score of 12 or higher indicates well controlled disease. DLQI: Dermatology Life Quality Index, a score of 6 or lower indicates no or small effect on quality of life.

Table 2:
PRO scores with regard to a) disease activity and disease control, and b) quality of life
a)

Moment	n	UAS7	n	UCT
Baseline	18	31.5 (25.8-38.0)	18	3.0 (2.0-6.25)
3 months	18	7.5 (0.0-22.0)**	18	12.0 (6.75-16.0)**
6 months	18	5.5 (0.0-17.3)**	18	11.5 (6.25-16.0)*
End FU	17	23.0 (11.5-30.0)*	16	7.0 (6.0-11.8)*

Data are shown as median scores (IQR). *statistically significant difference with baseline, by Wilcoxon signed rank test, p < 0.007. **statistically significant difference with baseline, by Wilcoxon signed rank test, p < 0.001. UAS7: weekly Urticaria Activity Score, UCT: Urticaria Control Test, AAS7: weekly Angioedema Activity Score.

b)

Moment	n	DLQI	n	CU-QoL
Baseline	18	11.1 (5.3)	18	34.0 (14.6)
3 months	18	3.3 (4.2)*	18	12.0 (11.6)*
6 months	15	3.0 (3.8)*	14	13.6 (10.2)*
End FU	17	5.8 (4.9)*	17	20.0 (10.8)*

Data are shown as mean scores (SD). *statistically significant difference with baseline, by paired T-test (p < 0.001 in all cases). DLQI: Dermatology Life Quality Index, CU-QoL: Chronic Urticaria Quality of Life questionnaire, AE-QoL: Angioedema Quality of Life questionnaire.

Inflammation characteristics in the skin prior to treatment

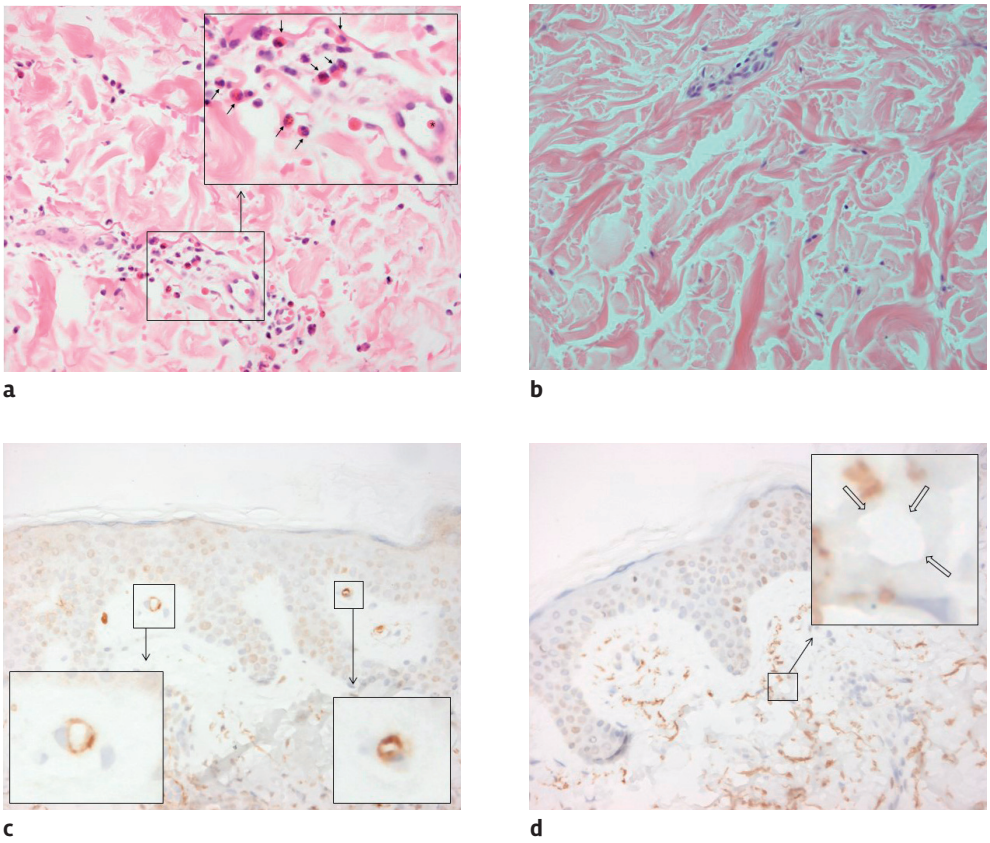
Edema was seen in lesional skin of 9 patients, perivascular infiltration in 14 patients, and interstitial infiltration in 7 (Table 3). Additionally, 14 patients showed elevated numbers of CD3-positive T-cells, elevated CD4-positive T-cells were seen in 16 and CD8-positive T-cells in 7. An elevated number of B cells was seen in 1 patient who also had signs of inflammation at the dermo-epidermal junction, and plasma cells in another, both had no dermatological comorbidities. Basophils were seen in only 2 biopsies. Non-lesional skin showed a smaller proportion of patients with edema, interstitial infiltration in the deep dermis, neutrophils and eosinophils compared to lesional skin, although not statistically significant (Table 3, figure 3ab).

Table 3: Inflammation and complement deposition in skin

	Lesional				**Non-lesional**			
Frequency of dermal changes	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Edema								
Superficial	9	8	1	0	13	4	1	0
Deep dermis	9	6	3	0	10	7	1	0
Perivascular infiltration								
Superficial	4	11	2	1	5	13	0	0
Deep dermis	15	2	1	0	17	1	0	0
Interstitial infiltration								
Superficial	14	3	1	0	16	2	0	0
Deep dermis	11	2	3	2	17	1	0	0
T-cells:								
CD3	3	13	1	0	3	13	2	0
CD4	2	12	4	0	2	11	5	0
CD8	11	7	0	0	12	6	0	0
B-cells:								
CD20	17	1	0	0	17	1	0	0
Plasma cells	15	1	0	0	16	1	0	0
Granulocytes								
Neutrophils	8	4	4	1	15	2	1	0
Eosinophils	11	2	3	2	16	0	0	2
Basophils	16	1	0	1	17	0	0	1
Mast cells	16	2	0	0	15	3	0	0
Histiocytes	4	9	5	0	6	12	0	0
C4d deposition	7	2	4	5	9	5	3	1

OMA; omalizumab. Score 0: not elevated, 1-3: mild, moderate or severe increase compared to healthy skin. In 9 biopsies it was not possible to score all items adequately due to technical errors, therefore not all characteristics add up to 18 patients.

Figure 3: 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

Complement activation in lesional and non–lesional skin

In baseline lesional biopsies, complement deposition (C4d) was seen in 11 patients (61%). In all 11 patients C4d deposition was present in walls of small blood vessels within the papillary dermis (Table 3). The intensity of C4d deposition was varying: In 5 patients, it was present in almost all vessels in the papillary dermis with a strong signal that surrounded the vessel walls fully (score 3 “strong”, figure 3c), in 4 patients C4d deposition was positive with a moderately strong signal or present in large portions of blood vessel walls (score 2 “moderate”), and in 2 patients it was present although the signal was very weak or it was only seen in small parts within a single blood vessel (score 1 “mild”). In the remaining 7 patients no C4d deposition was seen (score 0, figure 3d). Of the 11 patients with C4d deposition in lesional skin, 2 scored mild, 4 scored moderate, and 5 scored strong amounts. In non-lesional

skin only 9 showed C4d deposition including 5 who scored mild, 3 scored moderate, and 1 scored strong amounts (Table 3).-

For each of the 18 patients the amount of C4d deposition in lesional skin was compared to the amount in non-lesional skin. In 9 patients more C4d deposition was present in lesional skin compared to non-lesional skin. In 4 patients lesional skin showed less C4d deposition compared to non-lesional skin. The amount was equal in both biopsies from 5 patients. Notably, in three patients C4d deposition was seen in non-lesional skin but not in lesional skin.

In the total 36 skin biopsies, there was a trend that C4d deposition correlated with perivasculair infiltration in the superficial dermis (Spearman's ρ 0.353, p=0.035), eosinophils (Spearman's ρ 0.423, p=0.010), and neutrophils (Spearman's ρ 0.290, p=0.091). This suggests granulocyte infiltration is mediated by local complement activation.
The presence of C4d deposition in either lesional or non-lesional biopsies was not related to C5a levels in peripheral blood.

The two patients with elevated numbers of mast cells at baseline (patients 05 and 11) both did not show C4d deposition at baseline. Of the two patients with elevated numbers of basophils at baseline (patients 07 and 16), one showed C4d deposition.

Complement activation in peripheral blood before treatment with omalizumab

Table 4 shows that in most patients peripheral blood levels of all complement components investigated were within normal ranges throughout the study. 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 2 patients (11%) and elevated in 4 (22%), and throughout the study 18 (8%) C1q measurements were reduced and 28 were elevated (13%). However, as shown in Figure 4, C5a levels at baseline were increased compared to healthy controls (median controls 959,2 pg/mL, median baseline value patients 2415, p=0,0022). Among healthy volunteers, two outliers were seen (5%) whereas in the study population a larger proportion of patients were outliers (n=6, 33%). These results indicate complement activation in approximately one third of CSU patients. No correlations were found between complement component levels in peripheral blood and C4d deposition in skin.

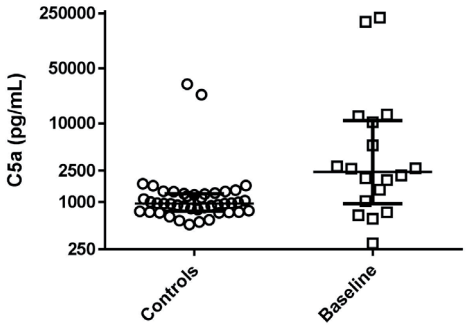


Figure 4: C5a levels at baseline

Data are shown as median C5a levels (IQR).

Table 4: Peripheral blood complement component levels and correlation with disease activity

		Baseline measurements		Total measurements		
Protein	**Normal value**	**Reduced n (%)**	**Elevated n (%)**	**Reduced # (%)**	**Elevated # (%)**	**Correlation with UAS7 ρ (p-value)**
C1q	81 – 128 IU/mL	2/17 (12)	4/17 (24)	18/224 (8)	28/224 (13)	0.298 (0.245)
C3	0.9 - 1.8 g/L	2/17 (12)	0	17/226 (8)	0	-0.064 (0.807)
C3bc/C3	n.a.	n.a.	n.a.	n.a.	n.a.	0.422 (0.091)
C4	0.1 - 0.47 g/L	2/17 (12)	0	14/226 (6)	0	-0.084 (0.750)
C4bc/C4	n.a.	n.a.	n.a.	n.a.	n.a.	0.269 (0.297)
C5a	<13605 pg/mL	n.a.	2/18 (11)	n.a.	12/252 (8)	-0.083 (0.744)
MAC	69 – 129%	1/18 (6)	1/18 (6)	13/249 (5)	12/246 (5)	0.154 (0.555)

Aberrant baseline column shows numbers and percentage of the patients (total 18 patients). Aberrant measurements column shows the number (#) and percentage of measurements aberrant from normal values at any time during the study. This includes the aberrant baseline measurements described in the Aberrant baseline column. For C3bc/C3 and C4bc/C4 no normal values are known and aberrant values could not be calculated. Correlation column: 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: 234 for C1q, C3, and C4, and 252 for C3bc, C4bc, C5a, and MAC. Number of missing values C1q:10, C3:8,C4:8,C5a:6,MAC:3. N.a.: not applicable, MAC: C5b-9 membrane attack complex formation. 'Reduced' and 'Elevated' indicate values below lower limit of normal, or above upper limit of normal. Numbers may not add up perfectly due to rounding.

Complement activation in peripheral blood after treatment with omalizumab

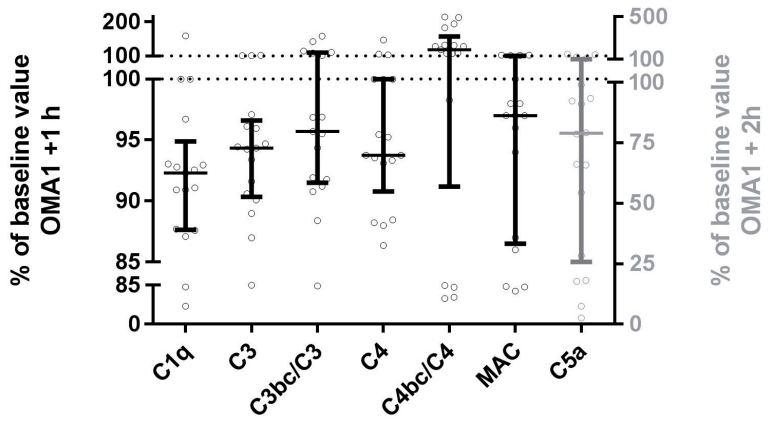
As shown in Figure 5, after the first omalizumab administration all but three patients showed an immediate decrease in complement components C3 (p=0.0002), C1q (p=0.0063) and a trend for decrease was observed in C3bc degradation products as shown after correction for total levels of C3, C4 and formation of the MAC complex, indicating that omalizumab administration leads to consumption of complement components. In addition, an increase in the and C4bc degradation products was found within an hour as shown after correction for total levels of C4. Finally, a decrease in C5a levels after 2 hours was found. After these time periods all concentrations of complement components and their degradation products returned to baseline levels.

At the second omalizumab administration no differences were found after two hours compared to the moment prior to administration. In addition, for all other time-points throughout the study, no statistically significant and clinically relevant differences were found compared to baseline or compared to the previous measurement.

Table 4 shows correlations between disease activity after 1 week and peripheral blood complement levels 1 hour after treatment. For all complement components, no significant

correlations were found. Additionally, no correlations were found between UAS7 after one week and both complement levels and difference in complement levels after one hour. Lastly, there was no relation between complement component levels 1 hour after treatment and the presence of treatment related adverse events throughout the study.

Figure 5: Response of complement to omalizumab administration



For each complement component median (IQR) and individual results are shown at 1 (black) or 2 (grey) hours after the first omalizumab administration as percentage of the baseline value. C3bc and C4bc activation ratios were determined by dividing the level of circulating C3bc or C4bc by the amount of C3 or C4 and multiplying the quotient by 100 to determine the percentage. OMA1: first omalizumab administration. MAC: Membrane Attack Complex.

Safety

As shown in Table 5, five patients (28%) reported at least one adverse event possibly, probably, or definitely related to omalizumab. No cases of anaphylaxis occurred. Two SAEs were reported during the study (1 case of abdominal bleeding and 1 case of dizziness leading to a prolonged observation time), both deemed unrelated to omalizumab.

DISCUSSION

This is the first study to demonstrate activation of the complement system in both peripheral blood and skin of CSU patients. We found complement deposition in skin in 61% of patients at baseline, and we found peripheral blood C5a levels at baseline to be elevated in approximately one third of patients, indicating complement activation in CSU patients. After treatment, complement components in the peripheral blood decreased within the first hour irrespective of disease activity. No relation was found between complement components investigated and disease activity (UAS7) scores prior to or after treatment with omalizumab, indicating that the early clinical effects of omalizumab cannot be explained by complement.

Table 5: Safety

Symptom	Frequency n (%)	Max. duration in days	Max. number of administrations*
Total number of patients reporting at least one related adverse event	5 (28)	28	5
Total number of related adverse events	10	28	5
Local adverse events	2 (11)	5	1
Dizziness	2 (11)	5	3
Malaise with nausea (and vomiting)	2 (11)	3	5
Headache	2 (11)	2	1
Joint and muscle pain or stiffness	1 (6)	28	3
Worsening of pre-existent fatigue	1 (6)	28	2

Side effects possibly, probably, or definitely related to omalizumab are shown. *For each patient the maximum number of administrations where the specific adverse event was recorded, is reported. Local adverse events included hematoma, and swelling (1 case each). Adverse events lasting up to the next administration are displayed as having a duration of 28 days.

In skin, complement deposition was seen in blood vessels of the majority of patients, indicating complement activation in skin of CSU patients. C4d deposition was shown in other autoimmune or inflammatory skin diseases, including deposition in blood vessel walls of urticarial vasculitis patients or systemic lupus erythematosus.[25] To our knowledge it is a novel finding that C4d is present in vessel walls in small blood vessels 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 pathogenesis of CSU.[5] Complement-fixing autoantibodies and complement deposition in the skin are also frequently found in systemic lupus erythematosus. In that disease, C4d was found not only in blood vessel walls (80% of patients) but also along the dermoepidermal junction (100% of patients). In subacute cutaneous lupus erythematosus deposits of C4d were shown within epidermal keratinocytes, and in pemphigus cases intercellular C4d was found which roughly corresponded to the location of autoantibodies.[25] The location of C4d deposition – in the superficial dermis – is rather similar to the location where infiltration was seen most in patients with urticaria. In these patients it is not surprising that C4d deposits were 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 be in fact post-lesional, since urticarial lesions tend to come and go – or it may point towards a systemic rather than local activation of the complement system. At present it is unknown whether C4d deposition in urticarial is limited to the skin. However, since symptoms in CSU are limited to the skin it would be difficult to measure C4d deposition in other tissues. The role of complement in CSU is further supported by the fact that baseline C5a levels in peripheral blood were elevated, which is also indicative of complement activation in approximately one third of CSU patients Since it is known that complement activation and in particular C5a can be important for basophil activation in urticaria,[9,26] 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, it was shown that after 13 days after clinical improvement C4d was still present in small traces, and only 21 to 41 days after clinical improvement a total clearance of C4d was found.[27]

Although not statistically significant, skin biopsies taken from wheals showed more neutrophils compared to non-lesional skin. This could be a response to local complement activation via C5a which was elevated in plasma. A difference in neutrophils in lesional versus non-lesional skin has been shown before, although in other studies other cell types could also differ between lesional and non-lesional skin[28] or between CSU skin and non-atopic control subjects.[29] This was not confirmed in the current study, possibly due to the low number of patients with inflammation in skin at baseline.

Complement activation at baseline may be caused by autoantibodies known to be commonly present in CSU patients.[5,30–32] Notably, we did not measure autoantibodies. 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.[33]

Upon omalizumab administration complement activation was found within an hour and normalization of C5a within two hours. Immediate response of the complement system has been shown before in rituximab treatment where complement consumption could be observed already within 5 minutes after completion of RTX infusion,[34] and an increase in C4bc was observed 30 minutes after onset of infusion.[35] Hence, this immediate complement activation is not specific to omalizumab. In addition, side effects of rituximab could be explained by complement activation early after infusion.[35] Although in the current study early activation was observed after administration of omalizumab we did not find a relation with occurrence of side effects. We also found that already in the second hour after omalizumab administration complement component levels returned towards their baseline levels, which was also found at the second omalizumab administration and was previously shown for C3bc levels in OKT3 treatment (a CD3 monoclonal antibody).[36] This likely is a result of production of influx from tissues or new complement factors by the liver. Furthermore, as C5a normalization was not persistent throughout the study we conclude that the early clinical responses observed after treatment are not due to resolvement of complement-mediated pathophysiology in CSU. Not surprisingly, since disease activity is measured once weekly, and

complement components restore within a few hours, no correlation between complement levels and disease activity was found. The question remains how this temporary complement activation upon anti-IgE therapy can be explained. One possibility is that omalizumab, being an IgG1κ antibody, forms circulating immune complexes with IgE, leading to temporary complement activation.[37] Another possibility is, since omalizumab and free IgE form immune complexes[38] they bind the low-affinity FcεRII (CD23). However, since the omalizumab binding site is positioned between binding sites of both FCεRI and CD23, it blocks interactions with both receptors making the latter unlikely.[39]

Baseline demographic characteristics were fairly similar to previous studies, and we expect that our results are generalizable to the general CSU population in need of third-line treatment. Inflammatory characteristics in skin appreciated the presence of dermal edema and perivascular and interstitial infiltration consisting of T-cells (mainly CD4-positive cells), and elevated neutrophils, eosinophils, macrophages, and in some patients mast cells. Although not specific for urticaria these findings typically characterize urticarial lesions histologically.[1] The low amounts of basophils and mast cells in skin biopsies is consistent with literature.[28,29] With regard to disease activity, upon treatment with omalizumab a strong improvement in median disease activity was seen, although some patients responded after one day whereas others only responded after 3 administrations. Additionally, two patients did not show improvement of UAS7 scores at any time during the study. Since the MID of the UAS7 is estimated at 9.5-10.5 points,[40] the MID of the UCT is 3 points,[41] and for DLQI the MID is 2.2-3.1,[42] the differences from baseline as reported in Table 2 are clinically relevant. For CU-QoL questionnaires the MID has not been established, hence it is unclear whether this difference is clinically relevant. After three and six months of treatment 44% and 50% of patients reached well controlled disease activity, respectively. A meta-analysis revealed that in five previous RCTs a UAS7 score of 6 or less was achieved in 55.1% of 749 patients treated with omalizumab 300 mg, and that the mean decrease in UAS7 scores ranged from 17 to almost 22 points.[15,43–47] These results appear slightly better compared to findings from the current study, although results of treatment with omalizumab in daily practice have been reported to be even better[3,48,49] which is confirmed by our own experiences (manuscript submitted). Adverse events in the current study occurred in a minority, were mostly mild and self-limiting, and all were known side effects. The findings from this study confirm the efficacy and safety of omalizumab 300 mg per month, and support the use of it as add-on treatment as recommended in guidelines.[1,2]

In conclusion, we found C4d deposition in skin and elevated C5a levels in peripheral blood, both indicative of complement activation in CSU patients. Skin biopsies taken from wheals showed significantly more neutrophils compared to non-lesional skin, also indicative of local complement activation via C5a. These data warrant further studies on autoantibodies causing

complement activation including their role in the pathogenesis of chronic spontaneous urticaria.

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CHAPTER 8:

EFFECTIVENESS OF OMALIZUMAB IN A DAILY PRACTICE COHORT OF ADULTS WITH CHRONIC SPONTANEOUS URTICARIA

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Submitted

ABSTRACT

Background

Efficacy and safety of omalizumab has been proven in chronic spontaneous urticaria (CSU). In randomized controlled studies, only data up to 6 months of treatment are available, and patients in clinical trials often differ from daily practice patients.

Primary objective

We assessed effectiveness of omalizumab in adult CSU patients in daily practice in terms of disease control, relapses, and side effects.

Methods

A monocenter prospective cohort study was performed. Patient-reported outcomes investigated effectiveness, defined as an urticaria control test (UCT) score ≥12. Relapse was defined as UCT <12 after initial effectiveness. Demographics, disease characteristics, side effects and (concomitant) treatment regimens were retrieved from patients' records.

Results

Fifty-two patients were treated with a median of 11 omalizumab administrations (range 4-38). Twenty-five (48%) were concurrently treated with long-term immunosuppressants. Omalizumab was effective in 49 patients (94%) after a median of 1 administration (range 1-5). Intervals between omalizumab administrations were successfully elongated in 33 (63%), 4 stopped omalizumab after 9-18 administrations after achieving remission. Relapse was observed in 30 (58%). In 10 patients omalizumab was up-dosed or the interval shortened yielding (temporary) effectiveness in 8. Side effects including headache, dizziness, malaise, fatigue, and hair loss, were reported by 38 (73%), in 19 (37%) repeatedly occurring at more than three administrations. Five discontinued omalizumab due to side effects.

Conclusion

Omalizumab was highly effective in daily practice. However, part of patients experienced relapse(s). Intervals could be elongated individually. Side effects occurred in a majority, but were only in a minority reason for omalizumab discontinuation.

INTRODUCTION

The efficacy and safety of omalizumab has been studied extensively in patients with chronic spontaneous urticaria (CSU).[1–6] This resulted in licensing of omalizumab by EMA (European Medicines Agency) in 2014.[7] The level of evidence for omalizumab treatment in CSU patients is high, due to randomized controlled trials (RCTs) of good quality.[8] However, treatment periods in these RCTs were limited – six months being the maximum. Additionally, efficacy is tested under ideal and controlled circumstances, whereas effectiveness is assessed under normal circumstances of healthcare practice.[9] Patients participating in clinical trials often differ from daily practice patients due to strict in- and exclusion criteria.[9,10] CSU patients in daily practice, for example, may have received treatment with immunosuppressants prior to omalizumab. Limited information is available about the effectiveness of omalizumab in such CSU patients.[11] More knowledge about daily practice results is required to provide additional insights with regard to the effectiveness and safety of omalizumab.

The primary objective of this study was to determine the long term effectiveness, in terms of disease control and occurrence of relapses, in adult CSU patients treated with omalizumab in daily practice. Secondary objectives were a) to evaluate effectiveness of omalizumab on tapering immunosuppressants, b) to assess effectiveness of adjusting dosage and/or interval of omalizumab injections on disease control, c) to define possible predictors of fast and slow response to treatment, based on clinical characteristics, d) to determine effectiveness of omalizumab in terms of disease activity and quality of life (QoL), and e) to evaluate safety of omalizumab.

METHODS

Design and population

This monocenter prospective cohort study investigated effectiveness of omalizumab in daily practice CSU patients aged 18 or above who were treated with omalizumab in the outpatient clinic of the University Medical Center Utrecht, the Netherlands, from June 2013 until August 2016. Inclusion criteria were CSU, age 18 or above, and treatment in daily practice with omalizumab for at least 4 administrations to be able to describe long term effectiveness. Patients suffering angioedema (AE) without wheals were excluded. Written informed consent was obtained from all participants (in accordance with ethics committee protocol numbers 15-386/C and 13-272).

Omalizumab treatment regimens and concomitant medication

CSU patients with insufficient response to antihistamines up to fourfold or combination therapy with H2 antihistamines and/or leukotriene receptor antagonists (LTRA) were treated with omalizumab. Prior to treatment with omalizumab, some patients had already received treatment with immunosuppressants including cyclosporine. Inability to taper immunosuppressants was also considered an indication to start omalizumab, although the

Urticaria Control Test (UCT, see below)[12] score would indicate good disease control, and the weekly Urticaria Activity Score (UAS7)[13] score, low or absent disease activity.

Prior to EMA licensing, treatment with omalizumab initially started with a dosage of 150 mg every 4 weeks and after licensing with 300 mg every 4 weeks. In case of ineffectiveness after three doses, omalizumab dosage was raised to 450 mg every 4 weeks. When this remained ineffective, the dose was raised further to 600 mg and if necessary the interval could be decreased to three weeks, and subsequently to two weeks. All administrations were followed by an observation time of two hours after the first three doses, and 30 min for subsequent doses. Omalizumab was administered by healthcare providers prepared to manage anaphylaxis.

Omalizumab was initially prescribed in addition to concomitant treatment and antihistamines up to fourfold in all patients. Continuation of antihistamines was actively encouraged. If possible, treatment with immunosuppressants was stopped or tapered prior to, or during treatment with omalizumab. In the case of prednisolone, tapering schemes were designed individually.

Primary outcomes: long term effectiveness and relapse

Outcome measures for the primary objective were (a) time to effectiveness, and (b) time to relapse. Effectiveness of treatment was defined as a UCT[12] score of 12 or higher after receiving treatment with omalizumab. The UCT was handed to all patients at every administration. Effectiveness after the first administration (i.e. UCT ≥ 12) was defined "fast response", in contrast to "slow response" which occurred after the second or subsequent administrations. Long term effectiveness, in these "fast" and "slow" responders, was expressed as the ratio of administrations with effectiveness to the total number of administrations.

A relapse was defined as a UCT score decreasing to below 12 at any time during treatment – after effectiveness had occurred previously. It was investigated whether relapse was associated with an attempt to elongate the interval between administrations, or to taper omalizumab dosages or other concomitant medication. After a relapse, new periods of effectiveness could occur.

Secondary outcomes

Data regarding demographics, the presence of angioedema and/or CINDU, disease duration, omalizumab dosages, intervals between administrations, and the use of concomitant treatment were retrieved from the medical records. Data regarding comorbidities and atopic diseases including allergic asthma, allergic rhinitis, and atopic dermatitis, were collected by a structured questionnaire with the instruction that only diseases confirmed by a medical doctor could be reported. If blood samples were available, total IgE titer and specific IgE

for common aeroallergens (ImmunoCAP Phadiatop, Thermo Fisher Scientific Inc./Phadia AB, Uppsala, Sweden) were measured. In case of symptoms after ingestion of a specific food, specific IgE for that food was tested additionally.

The dermatology-specific questionnaire Dermatology Life Quality Index (DLQI)[14], as well as the disease-specific Chronic Urticaria quality of life questionnaire (CU-QoL)[15,16] were used to evaluate quality of life (QoL). These questionnaires were handed to the patients at the first administration of omalizumab, after three months of treatment, and subsequently every six months. Additionally, disease activity was measured by evaluation of the UAS7[13] throughout the treatment with omalizumab.

Lastly, the presence of side effects was actively evaluated as open-ended question, results were recorded by a physician or (research) nurse at every administration.

Validation of questionnaires

The UCT and CU-QoL questionnaires these were translated into Dutch by forward-backward translation prior to use. and UCT results were approved by the original author, dr. K. Weller (Allergie Centrum Charité, Universitätsmedizin Berlin). Subsequently they were linguistically validated by pilot testing and validated. The Dutch CU-QoL questionnaire (total score) showed an excellent internal consistency (Cronbach's alpha = 0.922), good test-retest reliability (intraclass correlation coefficient [ICC] = 0.672, 95%CI 0.509 – 0.789), and strong correlation with DLQI (ρ=0.712, $p<0.001$), UAS7 (ρ=0.637, $p<0.001$), and UCT (ρ=-0.615, $p<0.001$). The Dutch UCT showed an excellent internal consistency (Cronbach's alpha = 0.91), excellent test-retest reliability after 1-3 weeks (ICC = 0.99, 95%CI 0.964 – 0.995), and strong correlation with DLQI (ρ=-0.696, $p<0.001$), and UAS7 (ρ=-0.727, $p<0.001$). UCT and CU-QoL were available for use from May 2014.

Analysis

Descriptive statistics were used to report patient characteristics, disease characteristics, and omalizumab treatment regimens.

Effectiveness and relapse were depicted in a heat map. Time to effectiveness was visualized by Kaplan-Meier curve. The ratio of administrations with effectiveness to the total number of administrations received were calculated per patient. To assess influence of atopic disease, presence of angioedema, and the use of immunosuppressants on the time to effectiveness, odds ratios including 95% confidence intervals were calculated.

Descriptive statistics were used to report patient-reported outcome (PRO) scores at baseline, and after 3, 6, and 12 months of treatment. UAS7-scores were analyzed at similar

time-points. PRO scores during treatment were compared to baseline by paired T-test or Wilcoxon signed rank test, and dichotomized scores by McNemar test.

Descriptive statistics were used to report side effects. Causality of side effects was judged by the investigators on a per patient per side effect basis. Only side effects that were possibly, probably or definitely related to omalizumab were included for analysis. Side effects leading to treatment discontinuation or hospital admission were reported irrespective of causality. All analyses were performed using IBM SPSS Statistics version 21.

RESULTS

Patient characteristics

A total of 69 CSU patients were treated with omalizumab of which 52 patients were included (Figure 1). Patient and treatment characteristics are shown in Table 1. Median age was 39.5 (range 19-75) and 39 (75%) were female. Of the 52 included patients, 37 (71%) suffered angioedema in addition to wheals, and 20 suffered CINDU in addition to CSU (39%). Median disease duration was 4.5 years (range 4 months to 32.6 years). Twenty-three (44%) reported some form of atopic disease by history. Prior to omalizumab, 37 patients (71%) received treatment with higher than fourfold dosages of antihistamines. Additionally, 37 (71%) had received long-term treatment with an immunosuppressant prior to omalizumab, and 25 (48%) at the first omalizumab administration. Six patients (12%) received omalizumab despite a UCT score of at least 12 at baseline, indicating well-controlled disease (Figure 2). Five of them used immunosuppressants and one used hydroxychloroquine. One patient previously treated with omalizumab 300 mg every 4 weeks elsewhere, commenced with 600 mg every 4 weeks.

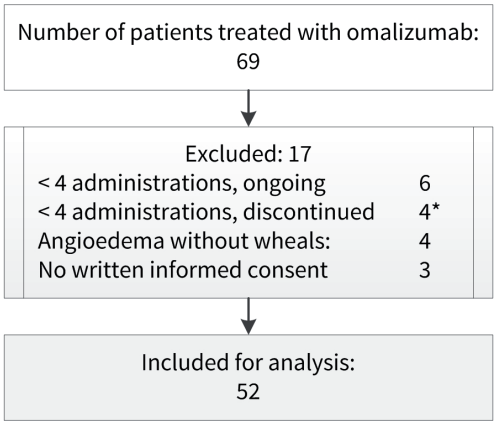


Figure 1: flowchart of in– and excluded patients

Patients who received less than 4 administrations were excluded since for those patients only short term information would be available. *Reasons for discontinuation within 4 administrations were on patient's request in all cases. They consisted of ineffectiveness (1 case) or side effects (3 cases).

Effectiveness and relapse during treatment with omalizumab

Figure 2 shows individual short term and long term treatment results. Median treatment duration with omalizumab comprised 11 administrations (range 4-38). Effectiveness occurred in 49 patients (94%) after a median of 1 administration (range 1-5). Twenty-eight (57%) had a UCT score of 12 or higher after one month of treatment (Figure 3a) and were classified

as fast responders. Eighteen other patients (35%) were slow responders. In 3 patients time to response was unclear because a baseline UCT score was missing, and in the remaining 3 no response was observed. Fast response could not be predicted by gender, atopic comorbidities, the presence of angioedema or CINDU, or the use of immunosuppressants at the time of the first omalizumab administration (Table 2).

Table 1: Characteristics of the 52 included patients

A. Patient and disease characteristics	n (%)
Median age in years (range)	39.5 (19-75)
Female, n (%)	39 (75)
Presence of angioedema, n (%)	37 (71)
Presence of CINDU, n (%)	20 (39)
Delayed pressure urticaria	14 (31)
Cold urticaria	2 (4)
Urticaria factitia	2 (4)
Median disease duration in years (range)	4.5 (0.33-32.6)
Median total IgE, in kU/L (range)	116 (<2-1036)
Atopy by history, n (%)	
Any atopy	23 (44)
Atopic dermatitis	8 (15)
Asthma	7 (14)
Allergic rhinitis	15 (29)
Food allergy	9 (17)
Sensitization in n=23 with positive history	
Aeroallergen screening (Phadiatop)	13 (57)
Birch pollen	6 (26)
Grass pollen	7 (30)
House dust mite	8 (35)
Food allergens as reported in history	2 (9)
Comorbidities, n (%)	
Hyperthyroidism	3 (6)
Hypothyroidism	3 (6)
Anaphylaxis	3 (6)
Adrenal insufficiency	1 (2)

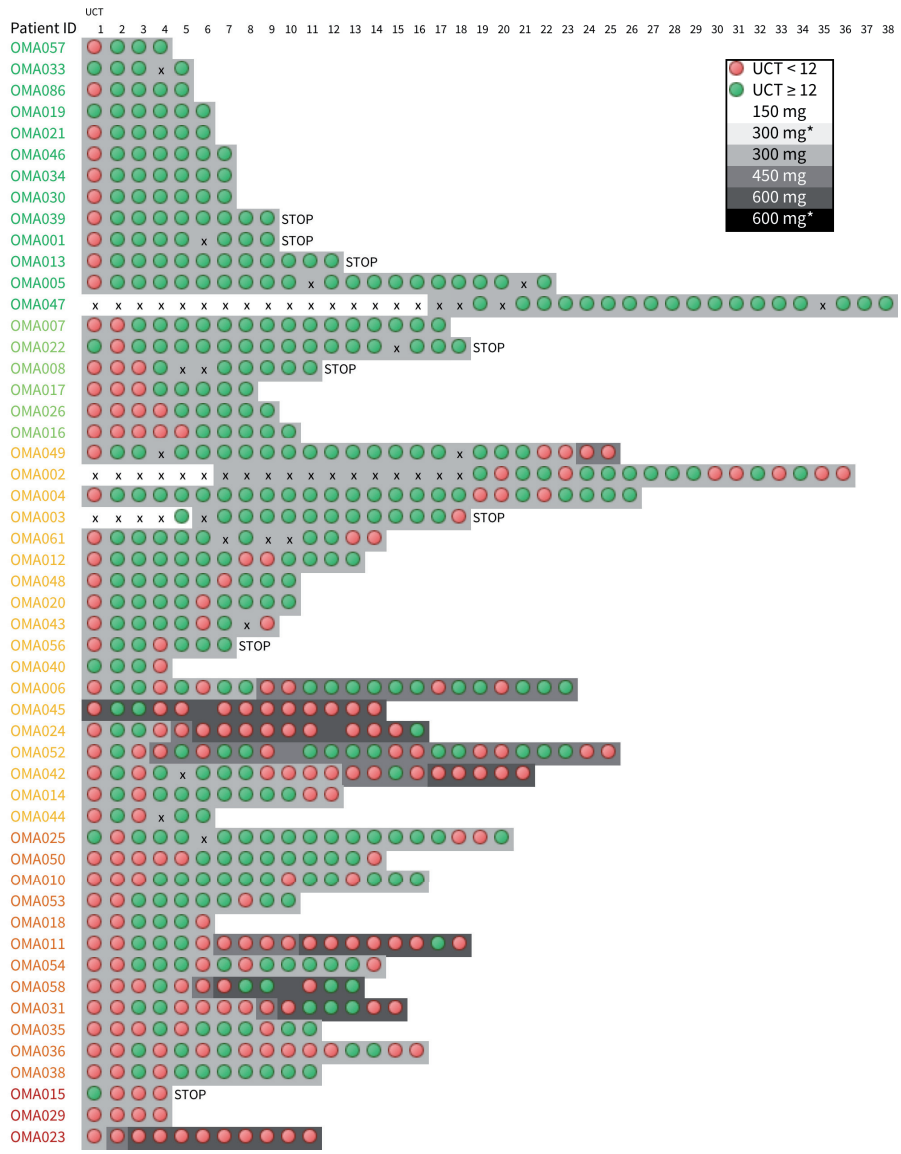
B. Treatment characteristics	n (%)
Concomitant treatment with antihistamines	52 (100)
Twofold	1 (2)
Threefold	3 (6)
Fourfold	10 (20)
Higher than fourfold	37 (71)
Treatment with first generation antihistamines	32 (62)*
Number of different antihistamines used	
2 types	6 (12)
3 types	6 (12)
4 or more types	40 (77)
Other therapies prior to omalizumab	
Prednisolone, short course(s)	27 (52)
LTRA	24 (46)
H2-antagonist	14 (27)
Hydroxychloqoruine	1 (2)
Indometacine	1 (2)
Gabapentine	1 (2)
Immunosuppressant prior to omalizumab	37 (71)
Prednisolone, long-term	24 (53)
CsA	27 (52)
MTX	8 (19)
Mycophenolic acid	2 (5)
Azathioprine	1 (2)
Any immunosuppressant at start of omalizumab	25 (48)
Prednisolone	14 (27)
CsA	11 (21)
MTX	1 (2)

Data are shown as numbers and percentages of patients for each characteristic, prior to treatment with omalizumab. Numbers may not add up perfectly due to rounding, in Table A since sensitization is shown as percentages of the 23 patients with a positive history, and in Table B because patients could use more than one type of medication simultaneously. Disease duration is shown prior to treatment with omalizumab. Adrenal insufficiency in one patient was a result of frequent use of prednisolone, prescribed to control CSU relapses. *all were additionally treated with second generation antihistamines. CINDU: Chronic inducible urticaria, LTRA: leukotriene receptor antagonists, CsA: Cyclosporine A, MTX; methotrexate.

Figure 4 shows the long-term effectiveness of omalizumab as a ratio of administrations with effectiveness to the total number of administrations, per patient. The median percentage of administrations with effectiveness was 67% (range 0-92). As shown, in 81% of the patients,

half or more of the administrations were effective, indicating that omalizumab was highly effective.

Figure 2: Effectiveness of omalizumab



Green dots indicate well controlled disease (UCT ≥ 12), red dots indicate poor disease control (UCT ≤ 11). Each dot represents a UCT score completed at one administration of omalizumab. Dosage and interval between administrations may differ between patients. Colors of the patient identification number indicate the time to effectiveness and long-term effectiveness: dark green: fast and maintained response, light green: slow, but maintained response, yellow: fast response with occurrence of relapse, orange: slow response and occurrence of relapse, red: no effectiveness after initiating treatment with omalizumab.

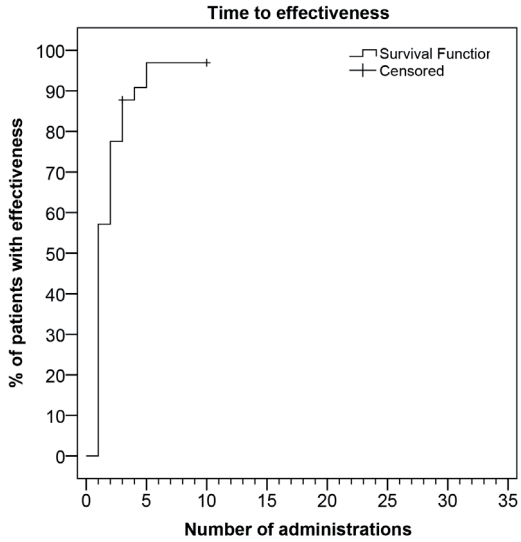


Figure 3: Time to effectiveness of omalizumab

Patients without baseline UCT score are excluded from analysis (n=3), therefore the total number of patients included is 49. In patients with a baseline UCT score of 12 or higher, the next administration with UCT ≥ 12 is shown as the moment of effectiveness.

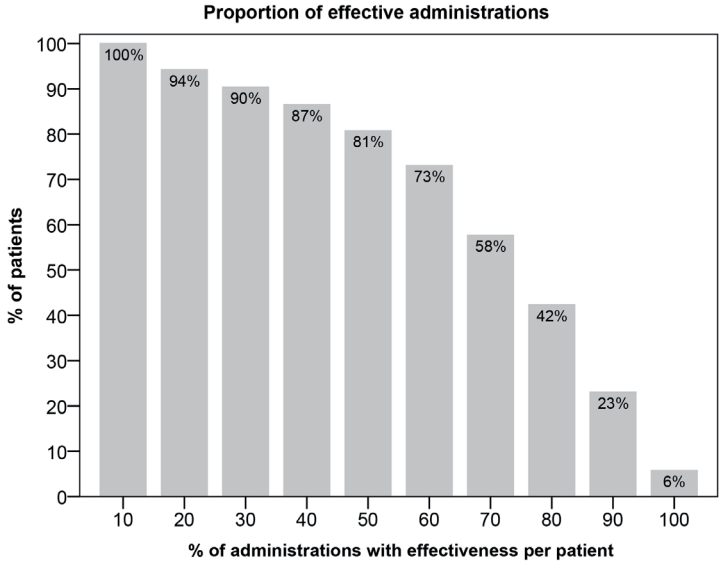


Figure 4: Proportion of effective administrations per patient

The long-term effectiveness of omalizumab is shown as a ratio of the cumulative number of administrations with effectiveness (UCT ≥ 12) to the total number of administrations, per patient. Each bar represents the number of patients with effectiveness in up to x% of administrations.

Relapse was seen in 28 patients (54%) after a median of 4 administrations (range 0-20) after reaching initial effectiveness. Two additional patients for whom the baseline UCT score was missing reported relapse, leading to a total number of 30 patients (58%). Relapse was seen after administration interval elongations in 12 patients (23%), concomitant treatment adjustments in 11 (21%), and a flare-up of comorbidities in 3 (6%). Lastly, 1 patient experienced side effects resulting in QoL impairment, and despite low disease activity this lead to a UCT < 12. In 3, no obvious association was identified. The occurrence of relapse could not be predicted by gender, atopic comorbidities, the presence of angioedema or CINDU, or the use of immunosuppressants at the time of the first omalizumab administration (Table 2).

Effectiveness of omalizumab on tapering immunosuppressants

Twenty-two out of the 25 patients (88%) on immunosuppressants were able to discontinue these, after a median of 3 omalizumab administrations (range 0-14). Two patients remained in need of corticosteroids and one in need of Cyclosporine A, although tapering was possible in two of these three patients. Furthermore, nine patients received one or more courses with of corticosteroids, one received montelukast, and in one patient methotrexate was prescribed in addition to omalizumab, because of insufficient results.

Effectiveness of adjusting dosage and/or interval

One patient started with 600 mg/4 weeks. In 9 patients omalizumab was up-dosed to 450 mg/4 weeks, yielding effectiveness in 3 who all experienced relapse (Figure 2). Of these three relapses, two were temporary and were followed by a new period of effectiveness. A total of 7 patients received omalizumab in 600 mg per administration. Effectiveness was seen in five out of 7, of which four experienced relapse. Of these relapses, 2 were temporary, and in two no further follow-up data was available. In 5 of 7 patients treated with 600 mg the interval was shortened, which led to effectiveness in 2 patients. In total, 8 of 10 patients experienced improvement on intensifying treatment, although (temporary) relapses often occurred.

Table 2: Possible predictors for fast or slow response and the occurrence of relapse

Characteristic	Fast n=28	Slow n=18	OR (95% CI)	No relapse n=18	Relapse n=28	OR (95% CI)
Gender: Male	7 (25)	4 (22)	1.13 (0.38-3.30)	3 (17)	8 (29)	0.58 (0.18-1.91)
Atopic disease	10 (36)	11 (61)	0.58 (0.32-1.09)	7 (39)	14 (50)	0.78 (0.39-1.55)
Angioedema	22 (79)	10 (56)	1.41 (0.90-2.23)	14 (78)	18 (64)	1.21 (0.84-1.75)
CINDU	8 (29)	8 (44)	0.64 (0.29-1.40)	6 (33)	10 (36)	0.93 (0.41-2.12)
Immunosuppr.	16 (57)	7 (39)	1.47 (0.76-2.85)	11 (61)	12 (43)	1.43 (0.81-2.51)

Data are shown as numbers and percentages. Patients without baseline UCT score and without effectiveness are excluded from analysis (n=6). Of the patients without baseline UCT score, two experienced relapse, and the number of patients with relapse therefore differs from the text. 'Atopic disease' shows patients with a positive history for atopic diseases. Immunosuppr: use of immunosuppressants at the time of initiating omalizumab, OR: odds ratio, 95% CI: 95 % confidence interval.

In patients with effectiveness, intervals between omalizumab administrations were successfully elongated in 33 cases (63%), with a median interval of 6 weeks (range 4.86-11.14 weeks) before symptoms started to recur. Four patients were able to discontinue omalizumab (after 9, 10, 12, and 18 administrations respectively).

At the time of data lock, treatment was ongoing in 44 (85%) patients. Reasons for discontinuation were pregnancy (n=1), inability to visit the hospital (n=1), and side effects (n=2, combined with ineffectiveness in one case) (see below).

Quality of life and disease activity

PRO results throughout the first year of treatment are shown in Table 3. DLQI, UAS7, and CU-QoL scores showed that after three months of treatment, a majority of patients (82%) had no or small effect on QoL (DLQI ≤ 6), a majority of patients (85%) had limited disease activity (UAS7 ≤ 6), and the mean QoL measured by CU-QoL decreased dramatically from 32.4 (SD 18.5) to 14.7 (SD 17.6). The differences with baseline scores were statistically significant for all three questionnaires, as were the results after 6 months of treatment. After prolonging treatment to 12 months, only CU-QoL scores showed a statistically significant difference.

Table 3: Disease control, quality of life, and disease activity

Moment	n	UCT ≥ 12 n (%)	n	DLQI ≤ 6 n (%)	n	UAS7 ≤ 6 n (%)	n	CU-QoL Mean (SD)
Baseline	49	5 (10)	48	18 (38)	18	5 (28)	50	32.4 (18.5)
3 months	46	32 (70)*	33	27 (82)*	13	11 (85)*	35	14.7 (17.6)*
6 months	38	31 (82)*	20	17 (85)*	11	9 (82)*	21	10.4 (14.0)*
12 months	20	14 (70)*	15	9 (60)	7	5 (71)	16	22.8 (25.3)*

Dichotomized UCT, DLQI, and UAS7 scores for each time-point were compared to baseline by McNemar test. Total CU-QoL scores for each time-point were compared to baseline by paired T-test. *statistically significant difference ($p<0.05$) compared to baseline. N.a.: not applicable, UCT: Urticaria Control Test DLQI: Dermatology Life Quality Index, UAS7: weekly urticaria activity score, CU-QoL: Chronic Urticaria Quality of Life questionnaire.

Side effects

Thirty-eight patients (73%) reported at least one side effect related to omalizumab (Table 4). The most frequently reported side-effects were headache, dizziness, and malaise, including flu-like symptoms. Side effects repeatedly occurring at more than three (subsequent or separate) administrations were reported in 19 patients (37%). Patients often reported that symptoms gradually decreased at each dose, and that side effects were generally mild. Three of 10 patients reported side effects to occur after up-dosing omalizumab, irrespective of the presence of side effects at the regular dose of 300 mg per 4 weeks.

Two patients reported hair loss at three or more administrations (Table 4). They reported this to last for 7 and 15 administrations respectively. Treatment was ongoing at time of data lock, and their treatment durations were 13 and 22 administrations, respectively.

No anaphylaxis occurred. In three patients, the observation time was prolonged due to side effects (one case with angioedema and dyspnea and two cases of instable asthma during the administration). In one patient (2%), abdominal bleeding caused by a ruptured ovarian cyst occurred after the 8th dose of omalizumab. This was deemed unrelated to omalizumab and she continued treatment with no recurrences. In total, five patients discontinued omalizumab due to side effects. This included three who discontinued treatment within 4 administrations. Reasons for discontinuation were 1) hair loss and arthralgia (discontinued despite effectiveness), 2) worsening of several pre-existent symptoms including depression, anxiety, hair loss, fatigue, gastro-intestinal complaints and paresthesia (no effectiveness), 3) hair loss, dizziness, and headache (patient was free of symptoms), and 4) angioedema after administration and headache and dyspnea one week later; this patient discontinued after the first dose (clinical effect unknown). No data were available regarding the fifth patient since informed consent was not obtained.

DISCUSSION

This study investigated individual and long-term effectiveness of omalizumab in daily practice. Effectiveness was seen in 94% of patients after a median of 1 administration (range 1-5). Fifty-seven percent were fast responders with effectiveness after the first administration. Fifty-eight percent experienced relapse after a median of 4 administrations (range 0-20) after reaching initial effectiveness. This included relapses after an attempt to elongate the interval between administrations. No predictors for fast or slow effectiveness, nor for relapse were found. It was possible to discontinue immunosuppressants in 22 out of 25 patients. Side effects were reported in 73% of patients – in half of them at no more than three administrations, and most were mild. Side effects were a reason for discontinuation of omalizumab in 5 patients, including three excluded patients.

Table 4: Side effects

Symptom	Frequency n (%)	>3 adm.* n (%)	> 3 days† n (%)
Total number of patients with at least one side effect	38 (73)	19 (37)	n.a.
Headache	21 (40)	9 (17)	6 (12)
Dizzyness or light-headedness	14 (27)	2 (4)	2 (4)
Malaise or feeling ill	11 (21)	4 (8)	6 (12)
Local symptoms	10 (21)	1 (2)	1 (2)
Fatigue	10 (21)	0 (0)	1 (2)
Worsening of pre-existent complaint	8 (15)	2 (4)	6 (12)
Hair loss	5 (10)	2 (4)	5 (10)
Pain in joints or muscles	5 (10)	0 (0)	4 (8)
Nausea and/or vomiting	4 (8)	1 (2)	0 (0)

Only side effects reported in three or more patients are shown. *Side effect repeatedly occurring at more than three (subsequent or separate) administrations. †duration of side effect reported to last longer than three subsequent days. Adm: administrations, n.a.: not applicable.

Individual responses showed periods of effectiveness and periods of relapse to be variable. Previously, efficacy of omalizumab was measured at a specific time-point such as 12 or 24 weeks.[1–6] Following such a procedure, and thus omitting many other time-points, will lead to a different proportion of patients experiencing effectiveness. In the current study both effectiveness as well as relapse were taken into account and it was therefore possible to obtain a complete overview of the long term response to omalizumab. Almost all patients (94%) experienced effectiveness, and more than half (57%) were fast responders. These results are consistent with one previous study describing daily practice and in one of the three pivotal RCTs.[3,18,19] This confirms that response to omalizumab in the real-world clinical setting in patients with CSU can be even better than that seen in the pivotal RCTs.[20] The longest interval between administration and manifestation of effectiveness was seen after 5 months of treatment, therefore confirming the importance of continuing treatment for at least this period of time. This was also concluded from the pivotal RCTs data.[19] Although the number of patients reporting relapse (54%) may seem high, in 12 of 30 patients relapse occurred after an attempt to elongate the interval between administrations. It is questionable whether these should be considered a true relapse as their inclusion leads to an overestimation of relapse occurrences. Furthermore, relapse was often followed by a new period of effectiveness. When these relapses are not taken into account, 18 patients remain, leading to a reduced relapse rate of 35%. It should also be noted however that some patients experienced more than one relapse during treatment.

A majority of the study population was refractory to antihistamines and even to immunosuppressants. Even in this severely refractory population 94% experienced effectiveness. Twenty-three patients (44%) had an atopic disease by history, and in 13 (57%) this was supported by sensitization to inhalant allergens. This is in line with literature.[23–26] In previous studies involving CSU patients receiving omalizumab, high percentages of sensitization up to 69% were reported – both in those with or without response.[24,26] Perhaps the presence of atopic diseases influences disease severity in CSU. Moreover, in the current study, patients with atopic diseases seemed to have a higher chance of slow response to omalizumab, although this was not statistically significant.

It was possible to individualize and extend treatment regimens in 33 patients (63%). In almost all patients treatment needed to be continued for a longer period of time, as shown in other studies.[18,20,27–30] Up-dosing was necessary in 10 patients (19%), and was effective in 8 (15% of total population). It was noted in a previous study that 16% of patients achieved complete disease control with 450 mg and a further 4% with 600 mg (personal correspondence, Giménez-Arnau. 2015).[20] However, we showed that (temporary) relapses often occurred and most patients did not achieve complete disease control after all administrations. Furthermore, the number of patients was low. Taken together, it remains to be determined

in which dosages and intervals treatment with omalizumab should be continued in patients with limited or no response.[20]

A majority of 38 patients (73%) experienced some side-effect during their treatment period, which is comparable to previous studies and to patients treated with placebo in the pivotal RCTs.[2–5,8,31,32] Side effects were reported in a minority after up-dosing, in accordance with other studies.[4,5,31] Most side-effects were mild and occurred only in the first few days after administration. Additionally, fatigue and hair loss were reported in 21% and 10% of patients, respectively, and these are not known to occur that often in comparison to other studies.[2–5,8,31,32] Notably, especially hair loss could be a reason for discontinuation of omalizumab.

In this study the UCT was used as the primary outcome. The most often used tool is the UAS7, which is recommended in guidelines.[33,34] However, patients need to complete the questionnaire prior to their first omalizumab administration and in the current study we faced missing baseline values for this questionnaire, reflecting the preference of patients for UCT instead of UAS7 in daily practice. Furthermore, different studies use different UAS7 cut-off values to evaluate effectiveness,[20] whereas the UCT has a clear cut-off value to discriminate between poor disease control and well-controlled disease.[12] The UCT is the first valid and reliable tool to assess disease control in patients with chronic urticaria – both spontaneous and inducible. Its retrospective approach and simple scoring system make it a valuable tool for the management of patients with chronic urticaria in routine daily practice.[12,35] The UCT correlates well with both UAS7 and CU-Q2oL scores,[35] and therefore it is unlikely that the use of the UCT has influenced the results of this study.

In conclusion, omalizumab was highly effective in refractory CSU patients. In the long term the proportion of administrations with effectiveness was high, although a part of patients experienced relapse at some time during treatment. Omalizumab afforded the possibility to taper immunosuppressants in almost all cases. Extending the interval was possible in almost two-thirds of patients experiencing effectiveness, and a minority were able to discontinue treatment. No predictors for fast response or for relapse were found. Up-dosing had temporary effectiveness. A majority had an improved QoL and improved disease activity after treatment with omalizumab. Side effects were mostly mild and transient, however, especially in the case of hair loss, can be a reason for omalizumab discontinuation despite effectiveness.

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CHAPTER 9:

GENERAL DISCUSSION

The research presented in this thesis focused mainly on treatment of adult patients with urticaria with or without angioedema, and patients with angioedema with or without urticaria. A reflection is given on the following themes that were assessed in this thesis: clinical characteristics of patients with angioedema with or without urticaria, the effectiveness and safety of treatment used in the past few years, thereby quantifying the need for additional treatment options, and currently available therapeutic options. We subsequently discuss effectiveness and safety results of additional treatment with omalizumab, in addition to the role of the complement system prior to and after treatment. In this thesis we distinguished chronic spontaneous urticaria (CSU) from angioedema without urticaria (idiopathic angioedema).

Nomenclature of angioedema without urticaria

In **chapter 2** we showed that angioedema without urticaria (idiopathic angioedema) is remarkably similar to angioedema with urticaria and angioedema caused by ACE-inhibitors (ACEi-AE) with regard to locations, frequency and severity of angioedema attacks. These findings suggest that the clinical presentation does not help to discriminate between these types of angioedema. However, the underlying mechanisms of these types of angioedema are probably different. Angioedema with urticaria is considered mast cell mediated, ACEi-AE is considered bradykinin-mediated, and in idiopathic AE the expected mechanism is unknown. There is an inconsistency in nomenclature in current guidelines on angioedema without urticaria (idiopathic angioedema), which adds to the diagnostic confusion. At least three guidelines describe angioedema.[1–3] Each guideline has its own classification and nomenclature, and each presents a different view on the suspected mechanism responsible for idiopathic angioedema. Consequently, recommendations for further diagnostic tests as well as for treatment differ among these guidelines, thereby challenging and confusing clinicians responsible for treatment of such patients. First, the current European guideline defined CSU as the appearance of wheals, angioedema, or both for a period of at least 6 weeks, due to known or unknown causes.[1] This means that according to the European guideline angioedema without urticaria – in this thesis referred to as idiopathic angioedema – should be diagnosed as CSU. As a consequence this guideline suggests that idiopathic angioedema is mast cell mediated.[1] Secondly, the current US guideline describes CIU (using the term chronic idiopathic urticaria (CIU) rather than CSU) and other forms of chronic urticaria separately from angioedema.[2]The treatment algorithm presented in the US guideline does not apply to patients suffering idiopathic angioedema in contrast to the European guideline. The US guideline considers idiopathic angioedema to be either bradykinin-mediated, mast cell mediated or unknown. Finally, there is a Hereditary Angioedema International Working Group (HAWK), which has recently developed a classification for angioedema without urticaria which proposes a different nomenclature. Here, family history and response to antihistamines are included in the classification. In case of a positive family history, and after the appropriate diagnostic testing, idiopathic angioedema is diagnosed as Hereditary

angioedema of unknown origin (U-HAE). In case of a negative family history, *ex juvantibus* treatment with antihistamines is recommended. Based on the response, idiopathic angioedema can be classified as Idiopathic histaminergic acquired angioedema (IH-AAE) or Idiopathic nonhistaminergic acquired angioedema (InH-AAE), with persistence of symptoms in the latter. The HAWK consensus considers IH-AAE to be mast cell mediated, and it considers a role for bradykinin, or for other mechanisms in InH-AAE.[3]

Since these inconsistencies between different guidelines have consequences for the expected mechanism to cause the angioedema that is referred to as idiopathic angioedema in this thesis, they also have consequences for the treatment that will be prescribed to these patients. We therefore performed a systematic review to provide a rational for the different treatment options for the several types of non-hereditary angioedema including idiopathic angioedema.

Treatment of angioedema without urticaria

In **chapter 3** the results of the systematic review show that for ACEi-AE, effective treatment options mostly consisted of drugs known for treatment of HAE including icatibant (a selective bradykinin B2 receptor antagonist)[4] and C1 esterase inhibitor (C1INH) concentrates,[5–7] whereas for angioedema with urticaria only omalizumab was described. Idiopathic angioedema was effectively treated with either drugs known for treatment of CSU – as prophylactic treatment – or drugs known for treatment of HAE –prescribed to treat acute attacks. As mentioned above, different available guidelines suggest different types of treatment for idiopathic angioedema since they allocate idiopathic angioedema to different expected mechanisms. The HAWK consensus partially overlaps with the European guideline since they both recommend second generation antihistamines as first-line treatment for these patients.[1,3] However, in case of unresponsiveness to treatment, the European guideline would advise to start add-on treatment with omalizumab, cyclosporine A, or antileukotriens,[1] whereas the HAWK consensus points toward drugs used in treatment of hereditary angioedema (HAE).[3] These conflicting advises may confuse clinicians rather than guide them. We suggest as treatment of idiopathic angioedema to start with second generation antihistamines, and up-dose these up to fourfold if necessary (Figure 1). In case of persisting symptoms, the recently developed Dutch guideline for treatment of CSU – which includes idiopathic angioedema – suggests to consider antileukotriens (montelukast), a switch of antihistamines, or oral corticosteroids. However, due to a low level of evidence we do not recommend montelukast in treatment of idiopathic angioedema. A switch of antihistamines could be considered. In addition, the US guideline opts for a switch to first generation antihistamines.[2] Courses of oral corticosteroids up to ten days may lead to a long term remission in chronic urticaria.[8] Based on expert opinion doses between 20 and 50 mg/day are required.[1] Corticosteroids could be used as rescue medication although a course of up to ten days is rather long and usually not necessary given the attack duration in idiopathic angioedema. If symptoms persist,

add-on treatment should be considered. Notably, suggested add-on treatment steps are based on expert opinion or extrapolated from evidence available for chronic (spontaneous) urticaria, hence the level of evidence is very weak. The Dutch guideline incorporates disease activity, disease control and quality of life before starting add-on treatment.[9] In idiopathic angioedema this might also be a worthwhile since patients with a low attack frequency or attacks of low severity might suffice with only rescue medication, whereas patients with a high attack frequency may benefit more from prophylactic treatment. HAE guidelines do not provide specific indications on when long-term prophylactic should be initiated because there is no agreement among experts.[3,10,11] In most European countries prophylaxis is considered based on the frequency of attacks and impact on quality of life, although there is a high variability in judgment of impact on quality of life.[10] In case of prophylactic treatment and when also considering costs of treatment, tranexamic acid would be preferred over omalizumab. Notably, chapter 3 shows that part of patients will remain symptomatic upon treatment with tranexamic acid. Omalizumab would subsequently be preferred. Due to a lack of evidence immunosuppressants are not included in the treatment algorithm. Patients with prophylactic treatment should additionally be provided with rescue medication since breakthrough attacks, including laryngeal, may occur with any prophylactic treatment.[10] For treatment of acute attacks chapter 3 showed that both icatibant and C1INH seem viable options, although tranexamic acid has also been described successful as rescue medication.[12] For treatment of life-threatening episodes the adrenalin-autoinjector should be considered.[3]

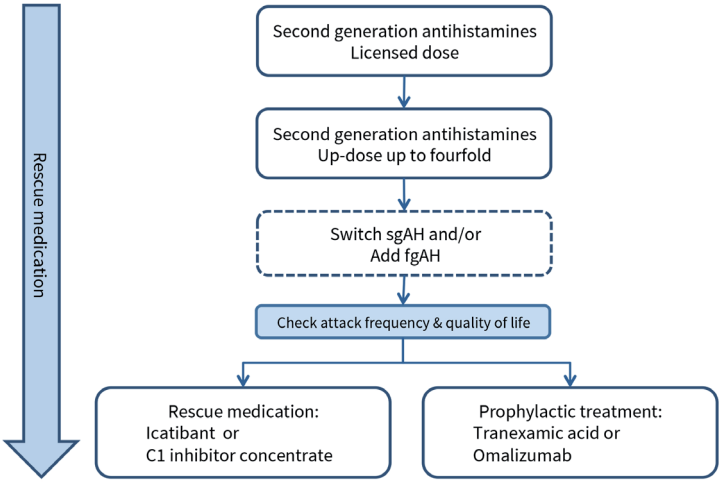


Figure 1: Proposed treatment steps for idiopathic angioedema

Patients should receive rescue medication during all treatment steps, including corticosteroids, tranexamic acid and/or adrenaline. Boxes with dotted lines indicate treatment options for consideration. Note that suggested treatment steps are based on very weak evidence or extrapolated from evidence available for chronic (spontaneous) urticaria.

Role of bradykinin and/or histamine in angioedema without urticaria

In **chapters 2 and 3** we conclude that bradykinin-mediated and mast cell mediated angioedema share clinical similarities, and both licensed HAE drugs and CSU treatments showed a beneficial effect in a substantial amount of patients. This raises the question whether patients may suffer more than one type of angioedema. It is known that symptoms in ACEi-AE may persist after discontinuation of ACEi, and it was previously speculated that such patients have 'hidden' idiopathic angioedema which is disclosed by ACEi.[3]

Additionally, findings from chapters 2 and 3 support a role for both bradykinin and histamine in idiopathic angioedema. This is further illustrated by a case of our own experience: an otherwise healthy male of 32 years old presented in 2012 with a history of recurrent swellings since 2004. He suffered weekly from swellings of the tongue, lips and/or cheeks. Swellings occurred unilateral and started early mornings during his sleep. Some attacks had caused problems with swallowing and speech, but he had never experienced dyspnea. During physical examination swellings were not observed although they were observed on photographs the patient had taken at home. No other skin abnormalities and no other symptoms were present. Additional history and investigations did not provide further clues, and the patient was diagnosed idiopathic angioedema. Treatment with antihistamines up to nine-fold did not change attack frequency although the intensity of attacks decreased. From the day tranexamic acid was added to antihistamine treatment no further attacks were reported. Tapering of either antihistamines or tranexamic acid led to an immediate recurrence of symptoms. In the long term it was possible to taper both tranexamic acid and antihistamine dosages, however, up to the last consultation in 2016 this patient remained both antihistamine- and tranexamic acid dependent. Although this case is an example of effective treatment, in chapter 3 several cases were presented with ineffectiveness of tranexamic acid. It was previously suggested that the different responses of patients with idiopathic angioedema to either tranexamic acid or immunosuppressants and biological agents such as omalizumab indicate the heterogeneity of this form of angioedema with regard to mediators involved in its pathogenesis.[3] The role of histamine in idiopathic angioedema is reflected by a beneficial response to antihistamines in many patients.[3,13,14] The involvement of bradykinin in idiopathic angioedema unresponsive to antihistamines has recently been confirmed in four patients during six attacks where in all attacks plasma bradykinin levels were elevated, whereas in remission and in healthy controls normal levels were observed.[15] The case presented above supports that both bradykinin and histamine are involved in pathogenesis of idiopathic angioedema. Additionally, evidence indicates that bradykinin and histamine interact. This might explain why half of patients with ACEi-AE reported pruritus as prodromal symptom (chapter 2) which would not be expected for a bradykinin-mediated swelling. It is known that there is interaction between the contact, complement, and fibrinolytic system,[13,16,17] and that there is interaction between these systems and mast cells and basophils. Histamine causes local vasodilatation and increased vascular permeability,

leading to extravasation of plasma proteins, contact of factor XII (FXII) with cell surfaces, and subsequent activation of the contact system.[18] Activation of the contact system leads to plasmin generation[13,17,19] and to C5a release,[13] which in turn leads to histamine release from mast cells and basophils.[18] Additionally, D-dimer is a fibrin degradation product which has been shown to be significantly higher in patients with CSU in comparison with healthy controls.[20] D-dimer levels decrease when disease activity decreases, and it has not been ruled out that d-dimers may induce degranulation of mast cells.[21]

Future perspectives for diagnosis and treatment of idiopathic angioedema

First, nomenclature regarding idiopathic angioedema (as defined in this thesis) needs consistency. We recommend that experts in the fields of urticaria and (hereditary) angioedema reach a consensus and define a nomenclature that is supported widely and that connects both fields.

Data so far indicate that both bradykinin and histamine can play a role in idiopathic angioedema. The response to treatment directed towards either the contact system or mast cells as shown in chapter 3 and the patient case reflecting our experience in daily practice, supports this. However, during a first visit in an outpatient or emergency setting the underlying mechanism of angioedema is usually not (yet) clear. Treatment is usually started before results of diagnostic laboratory tests are available,[13] and often such tests are not performed at all. Furthermore, suggested laboratory tests such as full blood count and CRP aim to clarify the existence or absence of underlying causes but do not differentiate between subtypes of non-hereditary angioedema.[1] Clinicians therefore remain in need of laboratory tests or biomarkers that confirm involvement of either one of these systems. Future studies should address this.

Additionally, we feel there is room for improvement in the increase of use of tools for disease severity in patients with angioedema of all subtypes, and there is a need for cut-off values to increase interpretation of these tools. The Angioedema Activity Score (AAS)[22] and the Angioedema Quality of Life Questionnaire (AE-QoL)[23] have been developed to measure disease activity and quality of life (QoL), respectively. For AAS no cut-off values for severity are available so far, although the minimal clinically important difference (MCID) for the weekly is available. For AE-QoL, the MCID was established in 2016 thereby increasing the interpretability of its results. Although AE-QoL is able to measure changes in QoL,[24] no cut-off values for the impact on QoL are available. However, the AE-QoL can be used in both HAE and non-hereditary types of angioedema.[23,24] Since both questionnaires have been developed only in recent years implementation in daily practice and in studies needs further improvement. In addition to possible future biomarkers this will make it easier to compare different studies in the future, to compare studies performed in different types of angioedema, for individuals

it improves comparison of results for different therapies, and provides guidance when treatment needs to be intensified.

Concerns about hypersensitivity reactions

Acute attacks of HAE are often treated with C1INH concentrates. One example of a C1INH concentrate is conestat alfa (Ruconest, Pharming), a recombinant human C1INH (rhC1INH) derived from milk from transgenic rabbits expressing the human gene for C1INH.[25–27] Although it is highly purified, rhC1INH contains a very small proportion (approximately 0.001%) of host-related impurities consisting primarily of milk or dander proteins.[5] These milk or dander proteins could trigger allergic reactions in patients with a preexisting allergy to rabbit dander, when exposed to rhC1INH. This is the reason why the consensus report from the Hereditary Angioedema International Working Group warns for a risk of anaphylaxis in this population.[3] In addition, the protein content of rabbit milk is approximately 14% of which about 65% consists of various caseins, and the remaining proteins are whey proteins.[28] Although cow's milk proteins differ from rabbit milk proteins, rabbit-milk impurities in rhC1INH may theoretically elicit an allergic reaction in patients with cow's milk allergy because of cross-reactivity.[27]

According to the EMA licensing rhC1INH is contraindicated in patients with a known or suspected allergy to rabbits. The following precaution is recommended: Before initiating treatment all patients should be tested for the presence of IgE antibodies against rabbit epithelium. Only patients who have been shown to have negative test results should be treated. IgE testing should be repeated once a year or after 10 treatments, whichever occurs first, and if symptoms of rabbit allergy develop.[5] In contrast, FDA prescribing information states that is contraindicated in patients with a history of allergy to rabbits, and also in case of allergy to rabbit-derived products. However, no pre-exposure or periodic testing is required.[29] Despite the fact that in the US no pre-exposure testing is performed there have been no further reports of allergic events including in our own study. This supports that also in Europe no pre-exposure or periodic testing could be considered.

In **chapter 4** we studied 22 patients with an allergy to rabbit and/or cow's milk who had a negative SPT and ICT result to rhC1INH, and none showed an immediate-type hypersensitivity reaction or allergic adverse events during a drug challenge with rhC1INH. This safety study was performed after one healthy control reported allergic symptoms upon the first and only infusion of rhC1NH (100 IU/kg). The subject did not disclose rabbit allergy. Symptoms started approximately 2 minutes after rhC1INH infusion and eventually progressed to sneezing, conjunctivitis, nasal congestion, facial edema, coughing, dyspnea (expiratory wheezing noted on pulmonary auscultation), and sinus tachycardia (120 beats per minute). Blood pressure stayed within the normal range. The subject was treated with salbutamol, prednisone, clemastine, and oxygen upon which the subject recovered within an hour. Upon re-evaluation

the subject reported allergy to rabbit and other pets, and subsequently an elevated IgE to rabbit was found (information retrieved from Pharming Technologies). At that time relatively few patients had been exposed to rhC1INH. Up to now there have been many more exposures and albeit contra-indicated in rabbit allergy it appears that anaphylaxis to rhC1INH is much less frequent then initially expected. Combined with the findings from chapter 4 showing that rhC1INH can be administered safely in the large majority of patients, this resulted in the EMA aligning with the FDA. So, in Europe pre- or post-exposure rabbit IgE testing is no longer required.[5]

Currently, recommended treatment of acute attacks of HAE consists of either icatibant, ecallantide, fresh frozen plasma, or C1INH.[10] Ruconest is not the only available type of C1INH. Two types of plasma-derived C1INH concentrates (pdC1INH) are available additionally – in the Netherlands only one is available. Although the half-life of rhC1INH is shorter than pdC1INH, there is no difference in clinical efficacy when C1INH activity levels are restored to the normal range.[30] However, advantages of pdC1INH over rhC1INH are that it can be used as prophylaxis and during pregnancy.[10] Taking into consideration that the European prescribing information for rhC1INH was recently adapted, this permits the use of rhC1INH in emergency settings alongside other treatment options.

Safety of the various treatment options is an important issue. In **chapter 3** we described various treatment options in non-HAE patients: icatibant, C1INH, fresh frozen plasma, methotrexate, tranexamic acid, and omalizumab. For all these options no allergic adverse events were reported. As mentioned, in **chapter 4** no allergic adverse events were reported for rhC1INH, apart from the reported reaction in the healthy control. In **chapter 5** we reported on treatment with antihistamines, and in **chapters 6, 7 and 8** we described treatment with omalizumab. Although safety was not the primary objective of these studies, no allergic adverse events were reported. This is of interest especially for omalizumab, because it is known that in asthma patients, although therapy with omalizumab is generally well tolerated, approximately 0.1–0.2% of patients have experienced anaphylaxis.[31–35] Omalizumab-associated anaphylaxis occurred within the first hour of injection in 70% of episodes, and 78% occurred within the first three injections.[31,36] However, anaphylactic reactions are also described after 6 or more hours after administrations,[33] and even after receiving omalizumab therapy over 60 administrations.[36,37] Since only 0.1-0.2% of patients treated with omalizumab experience anaphylaxis, the number of CSU patients treated with omalizumab in clinical trials is too low to rule out that in CSU omalizumab can cause anaphylaxis. However, based on available literature it appears omalizumab can be administered safely in the large majority of patients. The mechanism underlying omalizumab-associated anaphylaxis remains unclear.[36] It is also unclear which patients, if any, are at risk for developing an anaphylactic reaction upon omalizumab administration. One hypothesis suggests that antibodies of IgG or IgE isotypes reactive with omalizumab may be associated with the risk of anaphylactic reactions. However, in two independent studies of limited sample size, no antitherapeutic

antibodies were detected.[36,37] Another hypothesis suggests that polysorbate may trigger an anaphylactic reaction. Polysorbates are commonly used in pharmaceuticals, cosmetics and foods as solubilisers, stabilisers and emulsifiers. Polysorbate 80 is a non-specific mast cell activator, at least in non-human models,[38] and several anaphylactoid reactions in humans have been described upon administration of drugs containing polysorbate,[38–40] including in patients with anaphylactoid reactions after omalizumab administration, supported by a positive intradermal test to polysorbate.[37] Although polysorbate 20 instead of polysorbate 80 is an excipient in omalizumab vials,[41] it has not been ruled out that anaphylactic events to omalizumab occurred in fact due to a reaction to polysorbate.[36]

In case an allergy to rhC1INH is expected because of a rabbit allergy, a negative SPT and ICT virtually rules out allergy, as reported in chapter 4. In case an allergy to omalizumab is expected, a SPT (skin prick test; diluted 1:1,000, 1:100, 1:10, and undiluted) as well as an ICT with a concentration of 1:100000 (1.2mg omalizumab/ml) can be performed safely without producing an irritant response.[36,42] In drug allergy, skin testing is the most widely used method to determine sensitization.[43,44] Although drug provocation tests are the gold standard, it is used less often as it requires trained personnel and dedicated hospital facilities.[43,45] Furthermore, successful desensitization to omalizumab has been described in few asthma patients, who continued on omalizumab therapy for 12 months after desensitization without any reported adverse events.[35,46]

Treatment of CSU with antihistamines

In **chapter 5** we showed that by up-dosing antihistamines higher than fourfold, half of CSU patients reached sufficient treatment response while only a limited increase in side effects was seen, and the need for other third line therapies could be decreased considerably. This result might contribute to future guidelines.

The European and US guidelines with respect to chronic urticaria have been developed prior to licensing of omalizumab,[1,2] but the Dutch guideline was developed after licensing. Additionally, in development of the Dutch guideline adherence to the GRADE methodology was highest.[9,47] In the European guideline a modified version of GRADE was used.[1,48] As a result of this we feel that the Dutch guideline is currently the best guide. We propose to incorporate the findings from chapter 5. The Dutch guideline has a stepped care approach where clinicians can consider a couple of treatment options, including addition of montelukast and a switch of antihistamines, before continuing to third line therapies. It has to be realized that these are options with a low or very low quality of evidence, and some therapies were included in the guideline based solely on expert opinion.[9] Even for up-dosing antihistamines up to fourfold limited evidence is available, but it is supported by ample experience of urticaria experts all over the world. We state that up-dosing antihistamines higher than fourfold is of special interest for specific situations, for instance in patients who experience side effects

or have contraindications to currently proposed third line treatments. However, since we showed that up-dosing antihistamines higher than fourfold can impact half of patients in need of additional third line treatment, it could be considered for all CSU patients alongside montelukast and a switch of antihistamines.

The European guideline recommends to up-dose the following second generation antihistamines up to fourfold: cetirizine, desloratadine, fexofenadine, levocetirizine, loratadine, rupatadine, and bilastine[1] – the latter is not available in the Netherlands. These antihistamines have been studied in detail in urticaria, and have been shown effective in addition to a good safety profile. However, for some of them evidence is only available for lower dosages. Levocetirizine 5 mg and desloratadine 5 mg were studied up to fourfold.[9,49] Cetirizine 10 mg was studied up to threefold,[50–53] and rupatadine 10 mg up to twofold.[9,54–56] Fexofenadine 120 mg was studied up to fourfold, although some experts prescribe 180 mg up to fourfold (personal communication M. Maurer, Charité Berlin).[9,57,58] Furthermore, available studies sometimes have low numbers of treated patients (six to 439) and short treatment periods (5 hours to six weeks). A head-to-head comparison between different antihistamines was only performed for levocetirizine and desloratadine.[9,49] Although the European guideline does not include ebastine 10 mg in the second generation antihistamines that were studied in detail in urticaria,[1] its efficacy up to fourfold was shown.[59] The main concern of up-dosing is the occurrence of side effects, mainly somnolence. It is known that most patients do not report an increase in somnolence when antihistamine doses are increased.[60] Although no other studies have been performed on this topic, we expected that combining different antihistamines when up-dosing higher than fourfold would result in additional effectiveness while minimizing side effects.

Treatment of CSU with omalizumab

Chapter 6 revealed that in five previous clinical trials a weekly urticaria activity score (UAS7) score of 6 or less was achieved in 55.1% of 749 patients treated with omalizumab 300 mg, and that the mean decreases in UAS7 scores ranged from 17 to almost 22 points.[48,61–65] From the moment the meta-analysis was performed, two additional clinical trials have been published with respect to omalizumab in patients with CSU. One of these two studies specifically investigated CSU patients suffering both urticaria and at least four attacks of angioedema in the last six months. The proportion of patients with UAS7 of 6 or lower was not presented and a direct comparison with previous studies cannot be made.[66] However, 40% of patients were completely free of symptoms (UAS7 = 0) at week 12, which is comparable to our meta-analysis where 38.1% showed complete response.[48,66] The median number of angioedema-burdened days during the 28-week treatment period in the omalizumab group was 9, compared to 30 in the placebo group.[66] Of the second trial only a congress abstract was available. The proportion of patients with UAS7 of 6 or lower was not presented. However, the mean change

from baseline in UAS7 at day week 12 was -23.1 (SD 12.94) which is comparable to the highest improvement seen in previous studies.[48,61–65,67]

In **chapter 8** we showed that omalizumab was highly effective in daily practice even in refractory CSU patients. Almost all patients (94%) experienced effectiveness. A favorable response was seen after the first administration in 57% of patients, and in the long term the proportion of administrations with effectiveness remained high. This supports the available evidence that omalizumab should be included in the treatment algorithm for CSU.[1,2,9] Guideline recommendations are usually based on clinical trials, and in case of omalizumab these probably not reflect its full potential. It was previously suggested that response to omalizumab in the real-world clinical setting in patients with CSU could be even better than that seen in the pivotal RCTs,[68] and our experience in daily practice as shown in chapter 8 confirms this. Guidance to advise clinicians how to continue treatment with omalizumab in the long term, is lacking, and therefore we provide some suggestions below.

Response to treatment can be judged either as sufficient or insufficient. Although there is no consensus among experts when response is sufficient. In most studies a UAS7 score of 6 or lower was considered a sufficient response to treatment.[68] In chapter 8 we showed that the UCT is also a valuable tool in daily practice because of its retrospective approach, simple scoring system, and clear cut-off value of 12 or higher reflecting well controlled disease.[69,70]

In case of sufficient response we suggest to continue treatment for at least three further administrations, since most of the patients have longstanding disease and spontaneous remission within this period is unlikely. If beneficial results are maintained we propose to taper omalizumab by extending the interval between administrations.[9,68,71] We suggest to extend the interval by one week at every administration up to an interval of 8 weeks, as suggested in the Dutch guideline.[9] In the study presented in chapter 8 we adhered to this scheme and it was possible to extend intervals in 63% of patients. However, discontinuation of omalizumab was only possible in very few patients (8%), in line with literature.[68,71–75] Therefore, we propose to discontinue treatment if symptom control is maintained at an interval of 8 weeks, but a consultation at the outpatient clinic at 12 weeks after the last administration should always be provided to be able to intervene promptly in case symptoms recur.

On the other hand there are patients with insufficient response to treatment. In chapter 8 we show that effectiveness was sometimes seen only after a total of 5 months of treatment (dosage was not taken into account). Therefore it is important to continue treatment for at least this period of time before concluding that a patient has insufficient response. Post-hoc analysis from the pivotal RCTs confirmed this and even showed effectiveness starting only after 6 months of treatment.[76] In case of ineffectiveness, we up-dosed to 450 mg after at least 3 injections of 300 mg omalizumab with 4 week intervals, and if necessary we subsequently

up-dosed to 600 mg after at least one additional month. In case no response was obtained the interval was shortened to 3 weeks and subsequently to 2 weeks. By using this scheme, up-dosing appeared necessary in 19% of patients, and was effective in 80% (corresponding with 15% of the total population) while causing side effects in a minority. However, also in these cases (temporary) relapses often occurred and most patients did not achieve complete disease control. Up-dosing already after three months of treatment will make it impossible to define whether response could be obtained by continuing treatment at the same dose or by up-dosing. When 5-6 months is too long to wait for response in patients with severe urticaria, one could consider a short course of oral corticosteroids for temporary symptom relief. Notably, omalizumab is licensed as add-on treatment, and in all RCTs treatment with antihistamines was maintained. Although some cases have been presented where omalizumab remained effective while antihistamines were discontinued,[74] for economic reasons we think omalizumab should be given in addition to antihistamines at all times, and we prefer to taper omalizumab rather than antihistamines, once the disease is well-controlled. Additionally, since CSU has a detrimental impact on quality of life,[1,77] shared decision making is important especially in those with insufficient response.

Comparison of the two clinical studies with treatment with omalizumab

Although **chapters 7** (clinical study investigating the involvement of complement) **and 8** (daily practice) both describe treatment with omalizumab, clinical results from chapter 7 were presented rather briefly. For interpretation and comparison of clinical results of the two studies, extended information is needed. In chapter 7 both UAS7 and urticaria control test (UCT) questionnaires were used. To compare both studies we will first describe UCT results of chapter 7 (clinical study), with effectiveness defined as a UCT score of 12 or higher. Here, 14 patients (78%) reached effectiveness at any time during the 6 months of treatment, 4 (22%) within the first month, and in 6 (33%) the UCT score decreased again later during treatment, indicating poor disease control, which was named relapse. In chapter 8 (daily practice), effectiveness occurred in 94% of patients at any time during treatment. In 57% of the total population this was reached after one month of treatment. Relapse was seen in 54% patients. Although in both studies a majority of patients reached well controlled disease at any time during treatment, there is a clear difference in proportion of patients with response after the first administration (22% vs. 57%) and in those with a relapse (33% vs. 54%). Notably, in both studies patients initially received treatment with 300 mg per month. The differences in effectiveness and relapse raise the question whether the populations were comparable. In both studies age and gender distribution were comparable, as well as the proportion of patients with inducible symptoms and with angioedema in addition to urticaria. However, in chapter 7 a larger part of patients reported atopic disease caused by a larger proportion of patients with atopic dermatitis (chapter 7: 39%, chapter 8: 15%). Additionally, in chapter 8 more patients had been treated with immunosuppressants prior to omalizumab (chapter 7: 28%, chapter 8: 71%). In chapter 8 patients with atopic diseases seemed to have a higher

chance of slow response to omalizumab. It is possible to speculate that there were higher IgE titers in this group and consequently decreased effectiveness, given the relatively low dose of omalizumab used for treatment of CSU compared to the dose used in asthma. On the other hand, pre-treatment with immunosuppressants might result in higher sensitivity to omalizumab.

Another difference between chapters 7 and 8 is the proportion of patients reporting adverse events. In daily practice patients (chapter 8) 73% reported adverse events although in half of them at no more than three administrations. In chapter 7 only 28% of patients reported adverse events. As mentioned in chapter 6, when combining 5 RCTs 73.7% of patients reported any adverse event, but this was comparable to patients treated with placebo.[48,61–65] The absolute effect indicated that for every 1000 patients treated, adverse events will be reported by 32 more patients compared to placebo.[48] Therefore the difference between chapters 7 and 8 is likely a result of the limited sample sizes. Reported adverse events were comparable in both studies and comprised headache, dizziness, malaise, nausea, local symptoms, fatigue, and joint/muscle pain. In chapter 7 no hair loss was reported, which may also be a result of the limited sample size. In all patients, no serious adverse events related to omalizumab occurred, and no anaphylactic shock occurred. Results from both clinical studies in addition to the meta-analysis in chapter 6 support the use of omalizumab as add-on treatment.

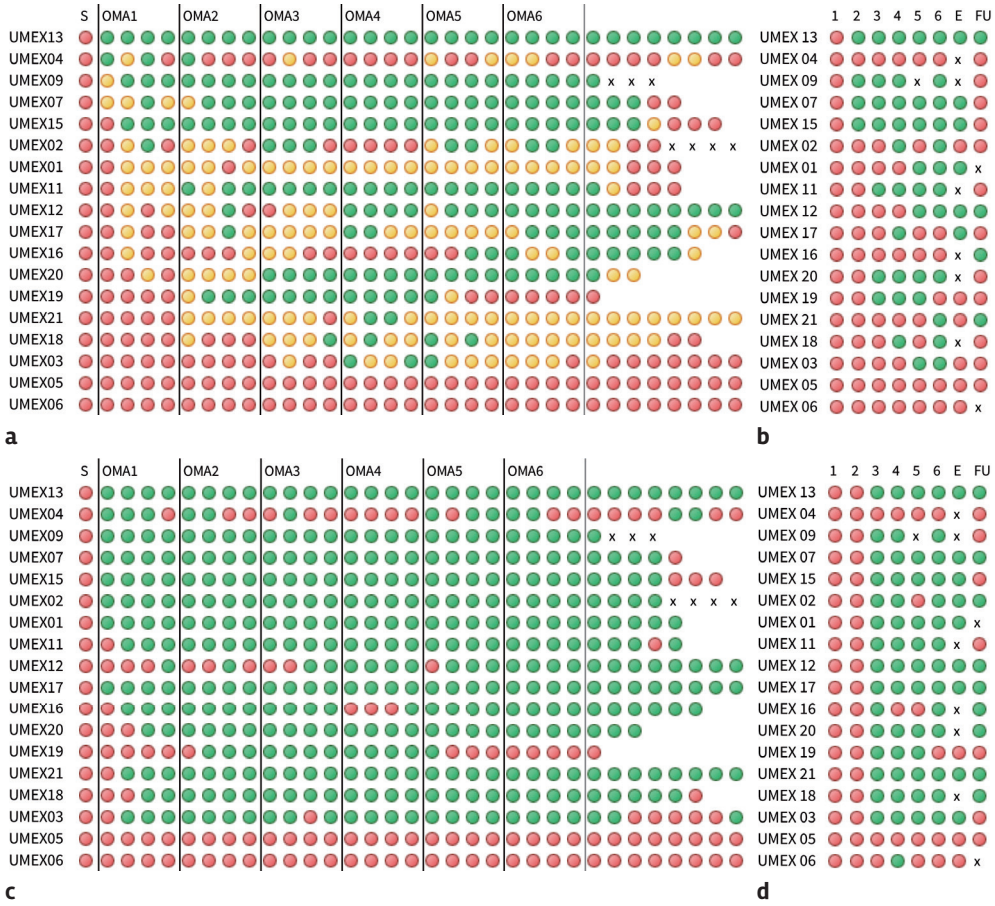
How to measure treatment response?

With a few examples we will demonstrate that the definition that is used to describe treatment response influences the proportion of patients with response. Figure 2 shows disease activity (UAS7, figure 2a) and disease control (UCT, figure 2b) from chapter 7 (clinical study). After three months of treatment 8 patients (44%) reached well controlled disease activity defined as a UAS7 score of 6 or lower. After six months of treatment this further increased to 9 (50%). In chapter 7, 15 patients (83%) showed a UAS7 score of 6 or lower at any time during the 6 months of treatment. Strikingly, when the full treatment period is taken into account and not only week 12, the proportion of patients with a UAS7 score of 6 or lower increases from 44% to 78%. This indicates that disease activity should be measured throughout treatment and it may even be the cause why response to omalizumab in the real-world seems better than that seen in the pivotal RCTs.[68]

An additional way to interpret clinical results is by incorporating the MCID (figures 2c and d). The MCID of the UAS7 is estimated at 9.5-10.5 points,[78] and the MCID of the UCT is 3 points.[79] Although the UAS7 scores often did not reach 6 or lower, and UCT scores did not reach 12 or higher, the vast majority of patients showed clinically relevant improvement. The MCID is generally not included in definitions for response, but we suggest to nuance results by including differences in MCID in addition to the raw values.

Lastly, in chapter 7 (clinical study) we used both UAS7 and UCT questionnaires. After three months of treatment a UAS7 score of 6 or lower was reported by 44%. At the same time a UCT score of 12 or higher was reported by 10 patients (56%). This shows that additional information is retrieved when both UAS7 and UCT questionnaires are used. This corresponds to the UCT content as it not only regards disease activity but also incorporates quality of life and treatment.[69] We suggest to use both questionnaires in evaluation of disease severity in studies and daily practice.

Figure 2: disease activity and disease control in the 18 patients from chapter 7



a) Individual UAS7 scores throughout the study. Red: UAS7 score 16 or higher indicating severe disease activity, yellow: UAS7 score 7-15 indicating moderate disease activity, green:UAS7 score 6 or lower, indicating low or no disease activity. S: screening, OMA: omalizumab administrations 1 to 6, x: missing. b) ndividual UCT scores throughout the study. Red: UCT 11 or lower indicating poor disease control, green: UCT 12 or higher, indicating well controlled disease. UCT 1 = filled out at administration no. 1 etcetera. E: end of treatment, FU: end of follow-up period, x: missing. c) UAS7 improvement of MCID (10 points) or more. Individual improvement of at least the minimal important difference (MCID; 10 points) in UAS7 scores was shown throughout the study, compared to baseline. Red: improvement less than MCID, green: improvement of at least MCID. S: screening, OMA: omalizumab administrations 1 to 6, x: missing. d) UCT improvement of MCID (3 points) or more. Individual improvement of at least the minimal important difference (MCID; 3 points) in UCT scores was shown throughout the study, compared to baseline. Red: improvement less than MCID, green: improvement of at least MCID. UCT 1 = filled out at administration no. 1 etcetera. E: end of treatment, FU: end of follow-up period, x: missing.

Mechanism of action of omalizumab

In chapter 7 we showed C4d deposition in skin, and elevated C5a levels in peripheral blood of CSU patients, both indicative of complement activation. After treatment with omalizumab an immediate short-lived further complement activation in peripheral blood was seen irrespective of disease activity which was not specific for treatment with omalizumab as the latter was also shown for rituximab and OKT3.80,81 We conclude that the early clinical responses observed after treatment are not due to resolvement of complement-mediated pathophysiology in CSU and that the exact mechanism of action of omalizumab remains to be elucidated.

Mast cells have always been considered to be the most important effector cell in CSU. Because it will take at least 1-2 months after treatment with omalizumab to have an effect on mast cells,[82] focus is now shifting towards analysis of other hematopoietic cells in the pathophysiological mechanism of CSU. A recent review shows that during treatment with omalizumab the number of FcεRI on basophils decreased by 11% after three days, by 78% after 10 days, and by 99% after 70 days in patients with allergic disease.[82–85] However, clinical response to omalizumab occurred within the first 2 days of treatment in at least half of CSU patients,[74,76] which cannot be explained by FcεRI down-regulation. Hence, especially in case of a fast response the exact mechanism of action of omalizumab remains unclear,[68] and combining cell numbers, FcεRI numbers, and activation potentials may provide more information. Chapter 7 is part of a broader project where this will be investigated at several time-points before and after initiating treatment with omalizumab in a total of 30 patients. We will investigate not only basophils but also examine other FcεRI-bearing leukocytes including monocytes and dendritic cells. Changes of inflammatory cell types can be related to changes in circulating complement levels, and to inflammation in skin, which may also provide new insights. This study is ongoing and results will be described in a separate manuscript.

Although the kinetics of down-regulation of the FCεRI on mast cells and basophils[86] cannot explain the early clinical effect of omalizumab, recently it was shown that omalizumab is able to dissociate IgE when bound to its receptor in basophils and mast cells, causing reduction of intracellular signalling by FCεRI within a few hours.[87,88] This early effect occurs prior to down-regulation of FCεRI expression and may explain the fast clinical response. Although the number of FcεRI on mast cells is reduced rather slowly, the potential to be activated ('releasability') may be influenced earlier.[83] Current *in vitro* evidence on this topic is conflicting as one study showed that omalizumab can inhibit mast cell and basophil activation[87] whereas in a different study omalizumab did not alter histamine release in mast cells or basophils.[89] This deserves further investigation.

Next to the fast clinical response, omalizumab provides a well-controlled disease in at least 50% patients during treatment. The mechanisms involved in this, besides down-regulation

of FCεRI expression, are not completely understood. Recruitment of basophils from circulation to the skin is relatively unique for CSU and an explanation for the prolonged effect of omalizumab may be that this recruitment is altered by omalizumab.[90] Furthermore, the expected pathogenesis of CSU includes the presence of IgG or IgE autoantibodies against TPO and –FcεRI. Antinuclear antibodies have also been described in urticaria although their fine specificity is unknown.[91] At present it is unknown whether the specificity or titer of these autoantibodies are related to the clinical phenotype of urticaria, but given the analogy with SLE, autoantibodies may lead to formation of complement-activating immune complexes. Is it possible that omalizumab influences the location of deposition and clearance of such immune complexes provided that they contain IgE?

Future perspectives for CSU and treatment with omalizumab

In the management of CSU patients, there is room for improvement by increasing the use of tools to objectify disease activity, disease severity, disease control, and quality of life. We showed that there is quite some influence on the results depending on which definition for response is used. We recommend that future studies use similar tools and outcome measures to allow better comparison among study results. Additionally, a definition for response to treatment should be developed which acknowledges that response may differ over time.

The mechanism of action of omalizumab in CSU is still not completely understood. Different mechanisms may contribute to the different effects of omalizumab (early versus late). This should be better understood, since it could help to predict responder types and enable a more patient tailored treatment approach. Given the rapidly changing clinical picture it would be of great help to have biomarkers for monitoring disease severity.[20] This may also help to predict which patients will respond to treatment, and to determine how long treatment should be continued.

The data above described the subgroup of CSU patients who all have urticaria. Patients with angioedema without urticaria were excluded in all studies. There is a need for further research regarding efficacy and safety of omalizumab in angioedema without urticaria, and additionally in children, and in chronic inducible urticaria. Another important remaining question is how to treat adult CSU patients with insufficient response to treatment with omalizumab. Such patients might be treated with immunosuppressants such as cyclosporine in the future, with or without omalizumab, given the favorable effect of omalizumab in patients that were previously treated with immunosuppressants. Other options may comprise TNF-α antagonists and rituximab (anti-CD 20).[92–94] Furthermore, new therapies and clinical studies are on their way and refractory CSU patients may benefit from therapies such as ligelizumab or quilizumab (anti-IgE), eculizumab (anti-C5a), prostaglandin D2 receptor antagonists, or specific inhibitors of molecules targeting intracellular pathways of mast cell activation following FceRI activation.[95]

Conclusions

With the research presented in this thesis we gained insights in treatment of adult patients with urticaria with or without angioedema, and patients with angioedema with or without urticaria. Our findings indicate a role for both bradykinin and histamine in idiopathic angioedema, which is supported by the available treatment options. We showed there is a need for third line therapy in more than half of CSU patients, which could be decreased almost by half when antihistamines were up-dosed higher than fourfold. We showed C4d deposition in skin and elevated C5a levels in peripheral blood of CSU patients, both indicative of complement activation. However, the early clinical responses observed after treatment with omalizumab are not due to an effect on complement. We confirmed the efficacy and safety of omalizumab. A favorable response was seen after the first administration in a majority of patients, and we showed long term effectiveness. Finally, we showed that omalizumab was highly effective even in treatment resistant CSU patients.

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CHAPTER 10:

APPENDICES

SUMMARY
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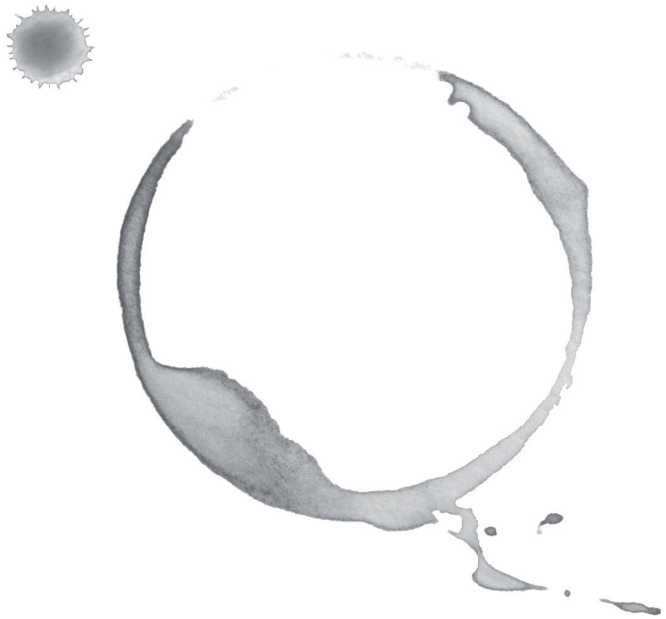
CURRICULUM VITAE



SUMMARY

The occurrence of wheals, angioedema or both for at least 6 weeks is diagnosed as chronic spontaneous urticaria in (inter) national guidelines - after excluding other illnesses. The underlying mechanism of angioedema without wheals is not entirely known. One possibility is that this is triggered by the same mechanism as (angioedema with) urticaria. Hereby, histamine is released from mast cells and basophiles, upon activation by IgG or IgE autoantibodies. Another possible cause can be found looking at the mechanisms underlying other hereditary variants of angioedema, in which the contact system plays a role. In the contact system, coagulation factor FXII is activated, thereby forming the enzyme kallikreine, which in turn cleaves high-molecular weight kininogen leading to formation of bradykinin. Bradykinin is the cause of edema in patients with hereditary angioedema, but also in angioedema caused by angiotensin I converting enzyme (ACE) inhibitors. In this thesis chronic spontaneous urticaria, including angioedema with urticaria is distinguished from angioedema without urticaria. The latter is further referred to as idiopathic angioedema.

The objective of this thesis is to increase insight in the treatment of adult patients with angioedema with or without urticaria, and with urticaria with or without angioedema. The thesis is divided into two parts that each have a different focus point. The first part addresses (non-hereditary) angioedema, with or without urticaria. In this part Chapter 2, 3 and 4 are included. In Chapter 2 the clinical characteristics of different variants of angioedema are presented. In our cross-sectional study, 104 patients with 1) ACE-inhibitor-induced angioedema, 2) angioedema with urticaria, or 3) idiopathic angioedema were included. The study showed that these three variants, despite their different expected mechanisms, are very similar in terms of location (on the body, on the skin?), frequency of attacks, disease severity and (prodromal) symptoms. This suggests that there may be a final common route leading to symptoms.

Chapter 3 is a systematic review of scientific papers on therapeutic options for different types of nonhereditary angioedema, when standard treatment with antihistamines, oral corticosteriods and/or adrenaline had insufficient results. From this review we conclude that acute attacks (in ACE inhibitor induced or idiopathic angioedema) can be treated effectively with icatibant, C1 esterase inhibitor (C1INH), or fresh frozen plasma. Response was often seen within 2 hours, with few and usually mild side effects. Prophylactic treatment (of idiopathic angioedema or angioedema with urticaria) consisted of omalizumab, tranexamic acid or C1INH. Effectiveness was seen in a majority of patients with limited and usually mild side effects. From this chapter we conclude that idiopathic angioedema can be treated effectively with drugs that involve a different pathophysiological mechanism. This also supports the suggestion that there might be a common route leading to symptoms.

In Chapter 4 we focus on the drug “Ruconest” (recombinant C1INH; rhC1INH), which is purified from rabbit milk from genetically modified rabbits. The medicine is used to treat

acute attacks of hereditary angioedema. Potentially, patients with an allergy to rabbit or cow's milk may also have an allergy to rhC1INH. In this chapter, we showed that in 22 patients with rabbit and/or cow's milk allergy and negative skin tests for rhC1INH (skin prick test and intracutaneous test), no allergic symptoms occurred during a subcutaneous challenge with rhC1INH. We concluded that rhC1INH is safe for the vast majority of patients with rabbit and/or cow's milk allergy and that negative skin tests virtually rule out an allergy to rhC1INH (negative predictive value 100%, 95% confidence interval 84.6-100%).

The second part of this thesis the focus is directed on chronic spontaneous urticaria with or without angioedema (according to the international and Dutch guidelines: CSU). In this part Chapter 5, 6, 7 and 8 are included. Chapter 5 describes a retrospective study of efficacy and safety of antihistamines in CSU patients. We showed that increasing antihistamines higher than four times the standard dose - fourfold is the maximum dose according to guidelines - was effective in 49% of patients, and it was associated with few and mild side effects. As a result, additional therapy in these patients had been avoided.

Since March 2014, the drug omalizumab has been registered as add-on therapy for CSU patients (12 years of age or older) with insufficient response to antihistamines up to four times a day. The results from a meta-analysis presented in chapter 6 confirm efficacy and safety of omalizumab 300 mg per month. Omalizumab is an antibody that binds to IgE. This leads to downregulation of IgE receptors on mast cells and basophils. However, it has not yet been fully elucidated how this leads to a reduction of symptoms. Downregulation of IgE receptors on mast cells and basophils occurs only after 1 week to > 2 months, while clinical improvement is often seen within a few days. Chapter 7 examined whether the complement system plays a role in this fast clinical response. In patients with CSU, prior to treatment with omalizumab both C4d was found in the skin as well as increased C5a levels in the blood, both indicative of complement activation. This is a new finding that suggests that autoantibodies play a role in the pathogenesis of urticaria. After omalizumab administration, apart from a very short-lived complement activation, no effect on the contact system was seen. We conclude that the complement system and factors that cause complement activation play a role in CSU. However, the rapid clinical improvement after omalizumab administration cannot be explained by complement. The exact mechanism of action needs to be studied further.

Finally, in chapter 8, we present the results of a study in which we investigated the effectiveness and safety of omalizumab in daily practice. In this study, 52 patients were treated with omalizumab with a median of 11 administrations (range 4-38). Effectiveness was seen in 94% of patients at any time during treatment, in majority of them even in the first month. Side effects were observed in 73% of patients. These were usually mild and were generally seen after three administrations or less. For some patients, side effects (especially hair loss)

were a reason to discontinue omalizumab. During treatment 3 patients had a spontaneous exacerbation; in 23 patients an exacerbation followed tapering of treatment of omalizumab (n=12) or concomitant treatment (n=11). In 4 patients an exacerbation was due to instable comorbidities or side-effects.Based on gender, atopy, presence of angioedema or inducible urticaria or the use of immunosuppressants during the first gift of omalizumab, we could not predict which patients would show rapid (<1 month) or late response, or who would develop a relapse. In the long term, effectiveness remained high in terms of disease activity and disease control, as well as quality of life. In case of ineffectiveness, a dose increase was effective in 8 out of 10 patients. In 63% of patients, the interval between two administrations could be prolonged, while effectiveness was maintained.

This thesis has increased insight into the clinical characteristics of angioedema with or without urticaria. It also provided an overview of therapeutic options for different types of non-hereditary angioedema, with or without urticaria. Antihistamines higher than four times the standard dose were effective in half of patients with CSU. Complement activation plays a role in CSU, but the beneficial clinical effect of omalizumab cannot be explained by an effect on the complement system. The efficacy and safety of omalizumab as described in literature was confirmed by a meta-analysis and by a study of its efficacy in daily practice.

NEDERLANDSE SAMENVATTING

Klinische kenmerken en behandeling van chronische urticaria en angio–oedeem

Het optreden van urticae, angio-oedeem of beide gedurende minimaal 6 weken wordt in (inter)nationale richtlijnen gediagnosticeerd als chronische spontane urticaria – na uitsluiten van enkele andere ziektebeelden. Het onderliggende mechanisme van angio-oedeem zonder urticae is niet zeker. Een mogelijke oorzaak is dat dit wordt veroorzaakt door hetzelfde mechanisme als (angio-oedeem met) urticaria. Hierbij komt histamine vrij uit mestcellen en basofiele granulocyten, doordat deze vermoedelijk worden geactiveerd door IgG- of IgE-autoantistoffen. Een andere mogelijke oorzaak kan worden gevonden in andere, hereditaire varianten van angio-oedeem en hierbij speelt het contactsysteem een rol. Hierbij wordt stollingsfactor FXII geactiveerd, waardoor het enzym kallikreine wordt gevormd, dat op zijn beurt zorgt dat het eiwit bradykinine wordt gevormd. Bradykinine is de veroorzaker van oedeem bij patiënten met hereditair angio-oedeem, maar ook bij angio-oedeem veroorzaakt door angiotensine I converterend enzym (ACE)-remmers. In dit proefschrift wordt onderscheid gemaakt tussen enerzijds chronische spontane urticaria, waaronder angio-oedeem met urticaria valt, en anderzijds angio-oedeem zonder urticae. Deze laatste vorm wordt verder aangeduid als idiopathisch angio-oedeem.

Het doel van dit proefschrift is om inzicht te vergroten in de behandeling van volwassen patiënten met angio-oedeem met of zonder urticaria, en van patiënten met urticaria met of zonder angio-oedeem. Het proefschrift bestaat uit twee delen. In het eerste deel ligt de nadruk op (niet-hereditair) angio-oedeem, met of zonder urticaria. In hoofdstuk 2 werd bestudeerd wat de klinische kenmerken zijn van verschillende varianten van angio-oedeem. In een cross-sectionele studie werden 104 patiënten geïncludeerd met ACE-remmer geïnduceerd angio-oedeem dat waarschijnlijk wordt veroorzaakt door bradykinine, angio-oedeem met urticaria dat waarschijnlijk wordt veroorzaakt door histamine, of idiopathisch angio-oedeem. Het onderzoek toonde aan dat deze varianten ondanks hun verschillende achtergrond erg vergelijkbaar zijn wat betreft locatie, aanvalsfrequentie, ernst en (prodromale) symptomen. Dit suggereert dat er mogelijk een gemeenschappelijke route is die leidt tot de klachten.

Hoofdstuk 3 is een systematische review waarin werd onderzocht welke therapeutische opties in de literatuur zijn beschreven voor verschillende typen niet-hereditair angio-oedeem, wanneer standaardmedicatie met antihistaminica, prednisolon en/of adrenaline onvoldoende effectief was. Hieruit bleek dat acute aanvallen (bij ACE-remmer geïnduceerd danwel idiopathisch angio-oedeem) effectief konden worden behandeld met icatibant, C1 esteraseremmer (C1INH), of fresh frozen plasma. Respons werd vaak gezien binnen 2 uur, met weinig en meestal milde bijwerkingen. Profylactische behandeling (van idiopathisch angio-oedeem danwel angio-oedeem met urticaria) bestond uit omalizumab, tranexaminezuur of C1INH. Effectiviteit werd gezien in een meerderheid van de patiënten, met weinig en meestal milde bijwerkingen. Uit dit hoofdstuk kunnen we afleiden dat idiopathisch angio-oedeem effectief kan worden behandeld met geneesmiddelen die een verschillend

pathofysiologisch mechanisme aangrijpen. Dit ondersteunt de suggestie dat er mogelijk een gemeenschappelijke route is die leidt tot de klachten.

Hoofdstuk 4 bestudeerde het medicijn Ruconest (recombinant C1INH; rhC1INH), dat wordt gezuiverd uit konijnenmelk van genetisch gemodificeerde konijnen. Het medicijn wordt gebruikt om acute aanvallen van hereditair angio-oedeem te behandelen. In theorie kunnen patiënten met een allergie voor konijn of koemelk ook een allergie hebben voor dit medicijn. In dit hoofdstuk lieten wij zien dat er in 22 patiënten met allergie voor konijn en/of koemelk en met negatieve huidtesten voor rhC1INH (skin prick test en intracutane test) geen allergische klachten ontstonden tijdens een subcutane provocatie met rhC1INH. We concludeerden dat rhC1INH veilig is voor de overgrote meerderheid van patiënten met allergie voor konijn en/of koemelk en dat negatieve huidtesten met grote waarschijnlijkheid een allergie voor rhC1INH kunnen uitsluiten (negatief voorspellende waarde 100%, 95%-betrouwbaarheidsinterval 84.6-100%).

In het tweede deel van het proefschrift ligt de nadruk op chronische urticaria met of zonder angio-oedeem (volgens de internationale en Nederlandse richtlijnen: CSU). Hoofdstuk 5 beschrijft een retrospectief onderzoek naar effectiviteit en veiligheid van antihistaminica bij patiënten met CSU. We lieten zien dat het ophogen van antihistaminica hoger dan vier keer de standaarddosering – wat de maximum dosering is volgens richtlijnen – effectief was bij 49% van de patiënten en gepaard ging met weinig frequente en milde bijwerkingen. Hierdoor kon aanvullende therapie bij deze patiënten worden vermeden.

Sinds maart 2014 is het medicijn omalizumab geregistreerd als aanvullende therapie voor patiënten met CSU van 12 jaar of ouder die onvoldoende respons hebben op antihistaminica tot viermaal daags. De meta-analyse in hoofdstuk 6 bevestigt effectiviteit en veiligheid van omalizumab 300 mg per maand. Omalizumab is een antilichaam dat bindt aan IgE. Dit leidt tot downregulatie van IgE-receptoren op de mestcel en basofiele granulocyt. Echter, het is nog niet geheel opgehelderd hoe dit leidt tot klachtenvermindering. Downregulatie van IgE-receptoren op de mestcel en basofiele granulocyt duurt lang (1 week tot > 2 maanden) terwijl klinische verbetering vaak al binnen enkele dagen gezien wordt. In hoofdstuk 7 werd onderzocht of het complementsysteem de snelle werking kan verklaren. Bij patiënten met CSU werd voorafgaand aan behandeling met omalizumab C4d depositie in de huid gezien evenals verhoogde C5a waarden in het bloed, wat beide wijst op complementactivatie. Dit is een nieuwe bevinding die suggereert dat autoantistoffen een rol spelen bij de pathogenese van urticaria. Na toediening met omalizumab werd behalve een kortdurende complementactivatie geen effect gezien van toediening van omalizumab op het contactsysteem. Wij concluderen dat het complementsysteem en factoren die zorgdragen voor complementactivatie een rol spelen in CSU. De snelle klinische verbetering na omalizumab lijkt echter niet te worden

veroorzaakt door een effect ervan op complementactivatie. Het exacte werkingsmechanisme moet nog verder worden opgehelderd.

Als laatste onderzochten wij in hoofdstuk 8 de effectiviteit en veiligheid van omalizumab in de dagelijkse praktijk. Daarbij werden 52 patiënten behandeld met een mediaan van 11 behandelingen (range 4-38). Effectiviteit werd op enig moment tijdens de behandeling gezien bij 94% van de patiënten en bij een meerderheid daarvan zelfs al in de eerste maand. Bijwerkingen werden gezien bij 73% van de patiënten. Deze waren meestal mild en werden gezien bij drie of minder toedieningen. Voor enkele patiënten waren de bijwerkingen (vooral haaruitval) een reden om de behandeling te staken. In het verdere verloop van de behandeling ontwikkelden 3 patiënten een spontaan recidief, bij 23 patiënten was er een recidief als gevolg van het afbouwen van de behandeling van omalizumab (n=12) of aanvullende behandeling (n=11) en bij 4 patiënten ontstond een recidief als gevolg van opvlamming van comorbiditeit of bijwerkingen. Het was op basis van geslacht, atopie, aanwezigheid van angio-oedeem of induceerbare urticaria of gebruik van immunosuppressiva bij de eerste gift omalizumab niet mogelijk te voorspellen wie snelle (<1maand) danwel late respons zou laten zien, of wie een recidief zou ontwikkelen. Op de lange termijn bleef de effectiviteit hoog zowel wat betreft ziekteactiviteit en ziektecontrole, als kwaliteit van leven. In geval van ineffectiviteit was ophogen van de dosering effectief bij 8 van de 10 patiënten. Bij 63% van de patiënten kon het interval tussen twee giften worden verlengd, zonder dat dit ten koste ging van de effectiviteit.

Dit proefschrift heeft het inzicht in het klinische beeld van angio-oedeem met of zonder urticaria vergroot. Ook heeft het geleid tot een goed overzicht van de therapeutische opties voor verschillende typen niet-hereditair angio-oedeem – met of zonder urticaria. Antihistaminica hoger dan viermaal de standaarddosering waren effectief bij de helft van de patiënten met CSU. Complementactivatie speelt een rol bij chronische urticaria, maar er is geen bewijs voor een gunstig effect van omalizumab via het complementsysteem. De effectiviteit en veiligheid van omalizumab, beschreven in de literatuur, werd bevestigd met een meta-analyse en door een studie naar de werkzaamheid ervan in de dagelijkse praktijk.

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LIST OF PUBLICATIONS

van den Elzen MT, van Os-Medendorp H, van den Brink I, van den Hurk K, Kouznetsova OI, Lokin AS, Laheij-de Boer AM, Röckmann H, Bruijnzeel-Koomen CA, Knulst AC. Effectiveness and safety of antihistamines up to fourfold or higher in treatment of chronic spontaneous urticaria. Clin Transl Allergy. 2017 Feb 14;7:4. DOI: 10.1186/s13601-017-0141-3.

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This article was selected to be highlighted in the Editors' Choice feature of the journal.

Urgert MC, van den Elzen MT, Knulst AC, Fedorowicz Z, van Zuuren EJ. Omalizumab in patients with chronic spontaneous urticaria: a systematic review and GRADE assessment. Br J Dermatol 2015;173(2):404-15. DOI: 10.1111/bjd.13845

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van den Elzen MT, Urgert MC, van Doorn MBA, Tupker RA, Knulst AC, van Zuuren EJ. Nederlandse richtlijn chronische urticaria – behandeling met omalizumab. Ned Tijdschr Dermatol Venereol 2015;25(3),134-136.

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CURRICULUM VITAE

Mignon werd geboren op 31 maart 1987 in Weert. In 2005 haalde ze haar Gymnasium diploma aan de Philips van Horne scholengemeenschap in Weert. In dat jaar begon ze met de opleiding Biomedische Wetenschappen aan de Universiteit Utrecht en na het eerste jaar verruilde ze dit voor de studie Geneeskunde. Tijdens het vierde jaar (2010) deed ze het co-schap gynaecologie en obstetrie in Kuala Lumpur, Maleisië. Tijdens het zesde jaar deed Mignon onder meer een onderwijsstage. Ook deed zij een keuzeco-schap dermatologie in het Zuwe Hofpoort ziekenhuis in Woerden onder begeleiding van Chris den Hengst met als verdiepingsonderwerp artritis psoriatica. Zij deed een wetenschappelijke stage bij de afdeling Dermatologie/Allergologie in samenwerking met het Laboratorium Klinische Chemie en Haematologie van het UMC Utrecht, onder begeleiding van André Knulst, Erik Hack en Coen Maas, met als onderwerp (hereditair) angio-oedeem. Ze haalde haar artsdiploma in november 2012 en begon aansluitend als arts-onderzoeker op de afdeling Dermatologie/ Allergologie van het UMC Utrecht, wat tot dit proefschrift heeft geleid. In 2017 is zij gestart met de opleiding tot dermatoloog in het UMC Utrecht, met Vigfùs Sigurdsson als opleider.