

Individually dosed omalizumab: an effective treatment for severe peanut allergy

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Summary

Background Treatment with omalizumab has shown a positive effect on food allergies, but no dosages are established. Basophil allergen threshold sensitivity (CD-sens) can be used to objectively measure omalizumab treatment efficacy and correlates with the outcome of double-blind placebo-controlled food challenge to peanut.

Objective To evaluate whether individualized omalizumab treatment monitored by CD-sens could be an effective intervention for suppression of allergic reactions to peanut.

Methods Severely peanut allergic adolescents ($n = 23$) were treated with omalizumab for 8 weeks, and CD-sens was analysed before and after. Based on whether CD-sens was suppressed after 8 weeks, the patients either were subject to a peanut challenge or received eight more weeks with increased dose of omalizumab, followed by peanut challenge or another 8-week cycle of omalizumab. IgE and IgE-antibodies to peanut and its components were analysed before treatment.

Results After individualized omalizumab treatment (8–24 weeks), all patients continued with an open peanut challenge with no ($n = 18$) or mild ($n = 5$) objective allergic symptoms. Patients ($n = 15$) needing an elevated omalizumab dose (ED) to suppress CD-sens had significantly higher CD-sens values at baseline 1.49 (0.44–20.5) compared to those ($n = 8$) who managed with normal dose (ND) 0.32 (0.24–5.5) ($P < 0.01$). Median ratios for Ara h 2 IgE-ab/IgE were significantly higher in the ED group (17%) compared to the ND group (11%).

Conclusions and Clinical Relevance Individually dosed omalizumab, monitored by CD-sens, is an effective and safe treatment for severe peanut allergy. The ratio of IgE-ab to storage protein Ara h 2/IgE as well as CD-sens to peanut may predict the need of a higher omalizumab dose. Clinical trials numbers: EudraCT; 2012-005625-78, ClinicalTrials.gov; NCT02402231.

Keywords basophil, food allergy, IgE, CD-sens, omalizumab (or anti-IgE), immunotherapy and tolerance induction

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Introduction

Foods that most commonly cause allergies in children are milk, egg, wheat, peanuts and tree nuts [1, 2], the latter two being the leading causes of anaphylaxis among children worldwide [3–5]. Despite the risk of

severe allergic reactions, there is to date no curative treatment for food allergy.

Recently, studies with different forms of immunotherapy for food allergy have been reported [6–10] and many are ongoing. In studies of peanut oral immunotherapy (OIT), the study subjects are heterogeneous with regard to the severity of their peanut allergy [7, 8, 10]. Also side-effects with allergic reactions are frequent [11, 12]. Further, anti-IgE treatment with

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omalizumab [humanized mouse monoclonal IgG antibodies (ab) which bind IgE] inhibits the allergic reaction in IgE-mediated allergy. Leung reported in 2003 promising effects of anti-IgE treatment for peanut allergy with the drug Hu-901 (never released on the market) [13]. The similar drug omalizumab is approved for allergic asthma and chronic urticaria but has shown positive effects also in food allergies [9, 14–17]. Omalizumab has also been used as a 'pre-treatment' for specific immunotherapy of perennial aeroallergens resulting in fewer systemic allergic reactions and more patients reaching the maintenance dose compared to placebo [18]. Interestingly, omalizumab was shown to significantly decrease adverse events in a study of milk OIT compared with milk OIT and placebo [9]. Besides, among peanut allergic children, pre-treatment with omalizumab before peanut OIT has shown to be effective [19]. However, omalizumab is an off-label treatment for food allergy, with no established dosages. Previous studies suggest that dosing should be individualized and that treatment effect has to be monitored [20, 21].

Basophil allergen threshold activation test is a method to objectively measure efficacy of omalizumab treatment. By triggering basophils *in vitro* with decreasing concentrations of allergen, the lowest triggering dose, the basophil allergen threshold sensitivity, CD-sens, can be determined. CD-sens correlates well with the outcome of double-blind placebo-controlled food challenge (DBPCFC) to peanut among children [22, 23]. In a retrospective study on peanut-sensitized adults, CD-sens correctly identified individuals with peanut-induced anaphylaxis [24]. Another study was able to differentiate patients with mild symptoms from patients who had severe reactions to peanuts [25].

The objective in this study was to evaluate whether omalizumab treatment, individualized in terms of dose and length of treatment, monitored by CD-sens and evaluated by peanut challenge, could effectively suppress allergic reactions to peanut.

Materials and methods

Study population

Severely peanut allergic adolescents, aged 12–19, were recruited in the Stockholm area. Inclusion criteria were evident history of peanut-induced anaphylaxis within the last 5 years, anaphylaxis or symptoms of an impending anaphylaxis as defined by WAO [26] at the open peanut challenge prior to inclusion (Table 3). Other inclusion criteria were IgE-ab > 0.35 kU_A/L to peanut and either Ara h 1, h 2 or h 3, and positive CD-sens and skin prick test (SPT) to peanut. Also, for a follow-up study, all patients had to have IgE-

ab > 0.35 kU_A/L, and a positive SPT, CD-sens and conjunctival provocation to an airborne allergen (pets or pollen) which served as a 'control allergen'. Serum IgE was required to be 30–1500 kU/L. Exclusion criteria were severe non-atopic chronic disease, pregnancy or allergy/hypersensitivity to omalizumab.

Baseline data also included medical history, physical examination, spirometry with reversibility test, exhaled NO-measurement and SPT.

Study design

This study is the first part in a two-step open phase 2 study of omalizumab treatment (first part) in combination with OIT (second part) in severe peanut allergy. The one-armed design was chosen for safety and ethical reasons. Omalizumab treatment was initiated according to the dosing scheme for allergic asthma, that is dose based on serum IgE concentration and body weight [27]. After 8 weeks, CD-sens was repeated. If CD-sens was negative or favourable (positive but with a non-calculable CD-sens value, see CD-sens section for definition), an open peanut challenge was performed during concomitant omalizumab treatment. If CD-sens was still calculable the patient received another 8-week cycle of omalizumab with a ~50% dose increment (available doses are 75 and 150 mg, and hence, exactly 50% increase was not always possible), followed by another CD-sens analysis. Depending on the CD-sens result, patients either continued with an oral peanut challenge or received another 8-week course of omalizumab at a further 50% higher dose. Omalizumab was continued and dose-increased until basophil analysis was negative or favourable (Fig. 1).

After the ensuing open peanut challenge, the second step of this study, peanut OIT under omalizumab protection was initiated in all patients. For the evaluation of OIT effect, all patients serve as their own controls by having a concomitant airborne allergy that will not be treated. Results from the second part will be presented separately.

The study was approved by the ethics committee in Stockholm; 2013/827-31/3, Swedish Drug Agency; 5.1-2013-46183 and registered at EudraCT; 2012-005625-78, ClinicalTrials.gov; NCT02402231. Patients and caregivers gave their written informed consent.

Peanut challenges

If patient history was not convincing concerning peanut-induced anaphylaxis < 5 years, an open peanut challenge was performed in hospital setting by experienced personal. Starting at 0.028 mg of peanut protein [0.1 mg of roasted peanuts with a protein content of 28% (Estrella, Angered, Sweden)], doses were increased

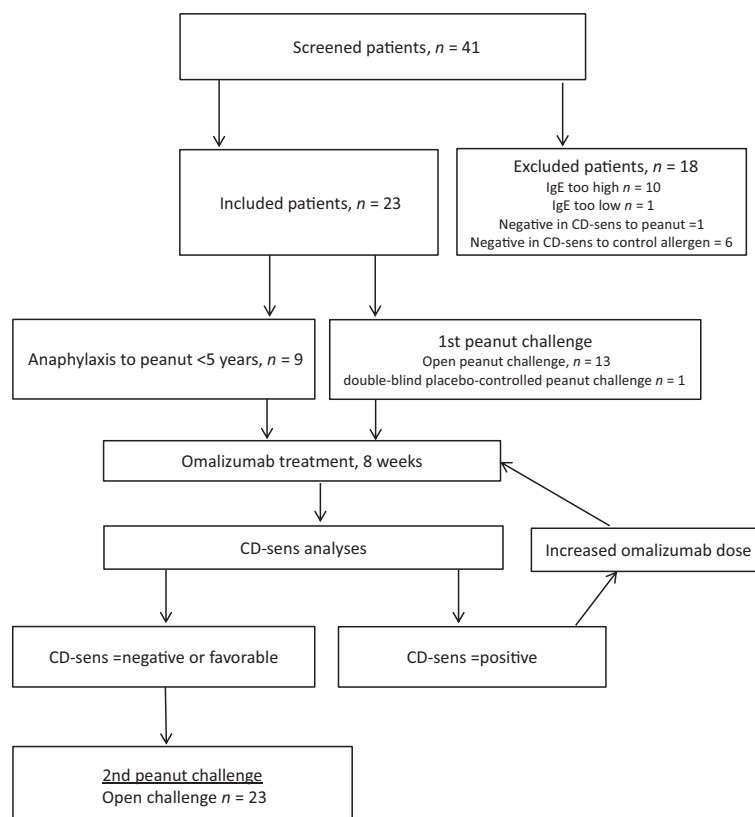


Fig. 1. Flow chart illustrating the study design.

every 30 min (0.28; 2.8; 28; 280; 2800 mg) the last dose equivalent to 10 g of roasted peanuts; however, individual dosing was used when the physician thought that the risk of very severe reactions was too high if going from, for example, 28–280 mg of peanut protein. Patients were observed for 2 h after the final dose or until all symptoms had resolved completely, whichever occurred last.

Peanut challenges during omalizumab treatment followed the same protocol as the challenge at inclusion. Symptoms were graded, using Sampson score [28].

Blood sampling

At screening, venous blood samples were drawn for CD-sens and IgE-ab analyses. CD-sens analyses were carried out within 24 h. After every 8-week cycle of omalizumab treatment, blood samples were drawn for CD-sens and serum was frozen in -20°C pending further analyses.

Basophil activation

Basophils were stimulated with decreasing concentrations of an in-house peanut extract (protein concentration range: 2.5–2500 ng/mL) [22, 29]. Anti-FcεRI (Bühlmann Laboratories AG, Schönenbuch, Switzerland) was used

as positive control and RPMI (cell culture media developed at Roswell Park Memorial Institute) as negative control. To differentiate the basophils from the leucocyte population, they were stained for CD203c. To detect activated basophils, the cells were stained for CD63 (Immunotech, Marseille, France) followed by analysis in a Navios flow cytometer (Beckman Coulter, Inc., Fullerton, CA, USA).

Cutoff determining a positive test was set to 5% of CD63-positive basophils. Patients, whose basophils, after stimulation with the positive control (anti-FcεRI) responded with < 5% CD63 up-regulation, were regarded as non-responders. Individuals with a response between 5% and 16% were classified as low responders. The cutoff of 16% was calculated (mean 76% – 3 SD) from the positive controls of an in-house reference material of 264 allergic children and adults [22]. CD-sens cannot be used to evaluate allergen sensitivity among non- and low responders.

Definition of CD-sens and 'favourable' test

To determine the basophil allergen threshold sensitivity, CD-sens, the eliciting allergen concentration giving 50% (EC50) of maximum CD63% up-regulation of the dose–response curve was calculated. CD-sens definition: the inverted value for EC50 multiplied by 100 [29].

When basophils react only at the highest allergen concentration, a CD-sens value cannot be calculated, nor can the test be ruled as negative. In this study, a positive, non-calculable CD-sens value was regarded as 'favourable'.

Serological analyses

IgE and IgE-ab to peanut and Ara h 1, Ara h 2, Ara h 3, Ara h 8 and Ara h 9 and to an airborne control allergen (timothy, birch, cat, dog or horse) were analysed using ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden), according to the instructions of the manufacturer.

Skin prick test

Skin prick test was performed with peanut extract (ALK, Copenhagen, Denmark). Histamine hydrochloride 10 mg/mL (ALK) was used as positive control and the diluent as negative control. The test was considered as positive if the wheal was ≥ 3 mm in diameter after 15 min.

Statistics

Continuous variables are presented as median and range if not stated differently. Binomial variables are presented as prevalence figures in the groups, respectively. For statistical comparison between groups, Fisher's exact test was used. Cutoff levels for CD-sens and IgE-ab/IgE ratios with optimal specificity and sensitivity were generated by calculating receiver operating characteristic (ROC)-curves. All statistical analysis was carried out using STATA 14.0 (Stata Corp, College Station, TX, USA). *P*-values < 0.05 were considered statistically significant. No corrections were made for multiple analyses.

Results

Patient characteristics

Of 41 screened patients, 23 fulfilled inclusion and exclusion criteria. Eighteen patients were excluded, and the main reason for exclusion was non-conformity to the IgE and weight-based dosing scheme for omalizumab [27] with too high ($n = 10$) or low ($n = 1$) IgE. Six patients did not show CD-sens reactivity to the control allergen, and one did not show CD-sens reactivity to peanut.

Median age was 17 (12–19) years and 30% were males. The majority (87%) had asthma ($n = 20$) and rhino conjunctivitis ($n = 20$). All were allergic to pets or pollen. Median IgE-ab level to peanut was 86 (range 30–350) and to Ara h 2 58 (16–220) kU_A/L. All participants had a positive SPT to peanut, median wheal size

10 (5–15) mm in diameter and positive CD-sens to peanut, median 0.75 (0.24–20.5) (Tables 1 and 2).

Nine patients had a convincing history of peanut-induced anaphylaxis < 5 years before, and they entered the study without going through a food challenge. One patient had an anaphylaxis in a DBPCFC to peanut in a previous study [22]. Thirteen patients without evident anaphylaxis < 5 years went through an open peanut challenge. Symptoms from this challenge or patient histories of worst anaphylactic reaction during last 5 years are presented in Table 3. All reactions were severe, mean Sampson score 3.8 (range 3–4). Nineteen of twenty-three (83%) received one or more injections of epinephrine. Four adolescents who went through the inclusion peanut challenge were hospitalized; two of them had bi-phasic reactions.

Dosage of omalizumab

Omalizumab was dosed according to the dosing scheme for allergic asthma, that is based on serum IgE level and body weight. After 8 weeks of omalizumab treatment, CD-sens was analysed and was negative/favourable in eight (35%) of the patients. These eight patients, referred to as the normal dose (ND) group, subsequently continued with an open peanut challenge. The remaining 15 patients (65%) received an elevated omalizumab dose, referred to as the elevated dose (ED) group. The protocol suggested an increased dose of 50%, up to the maximum dose of 600 mg every 14th day. One patient (ID 1) did by mistake receive a 100% dose increment (Table 3).

After 16 weeks on omalizumab, including increased dose, 12 of the 15 patients (80%) in the ED group had a negative/favourable CD-sens while three did not (ID 4, 6, 19). ID 6, who had reached the maximum dose of omalizumab, received eight more weeks with unchanged dose (in total 24 weeks) and was then barely positive in CD-sens. ID 4 and ID 19 also continued for another 8 weeks and received a further 50% dose increment. ID 4 was negative in CD-sens at week 24. ID 19 went through another 8-week cycle, with an additional 50% increased dose of omalizumab, in total 32 weeks, and thereafter had a favourable CD-sens (Table 4).

The starting dose differed significantly between the groups, 450 mg/4 week in the ED group and 675 mg/4 week in the ND group ($P < 0.027$). In contrast, when the omalizumab dose was adjusted for body weight and IgE, the median dose in mg/kg body weight/IgE IU/L was significantly higher in the ED group at baseline: 0.026 (0.018–0.056) as compared with 0.022 (0.017–0.024) mg/kg/IgE in the ND group ($P = 0.028$). This weight and IgE-adjusted dose increased to 0.043 (0.022–0.114) mg/kg/IgE for the (ED), as they reached negative or favourable CD-sens (Tables 1 and 3).

Table 1. Patient characteristics and baseline data

	All <i>n</i> = 23	Normal dose <i>n</i> = 8	Elevated dose <i>n</i> = 15	<i>P</i> -value
Gender, male, <i>n</i> (%)	7 (30)	1 (13)	6 (40)	0.35
Age, years	17 (12–19)	16 (12–18)	17 (13–19)	0.61
Weight, kg	58 (39–72)	53 (39–66)	61 (45–72)	0.11
Atopic co-morbidity				
Asthma, <i>n</i> (%)	20 (87)	7 (88)	13 (87)	1
Other food allergy <i>n</i> (%)	14 (61)	4 (50)	10 (67)	0.67
Eczema, <i>n</i> (%)	6 (26)	3 (38)	3 (20)	0.62
Allergic rhinitis, <i>n</i> (%)	20 (87)	7 (88)	13 (87)	1
Allergic conjunctivitis, <i>n</i> (%)	21 (91)	8 (100)	13 (87)	0.53
Allergy to furry pets, <i>n</i> (%)	18 (78)	7 (88)	11 (73)	0.62
Allergy to pollen, <i>n</i> (%)	20 (87)	7 (88)	13 (87)	1
> 2 atopic diagnoses, <i>n</i> (%)	15 (70)	6 (75)	9 (60)	0.66
IgE, kU/L	470 (81–860)	660 (500–790)	260 (81–860)	< 0.01
IgE-ab, kU _A /L				
Peanut	86 (30–350)	167 (35–260)	75 (30–350)	0.22
Ara h 1	21 (0.01–96)	20 (0.01–90)	21 (5.6–96)	0.77
Ara h 2	58 (16–220)	71 (26–102)	38 (16–220)	0.087
Ara h 3	3.4 (0.01–43)	5.35 (0.01–37)	3.1 (0.5–43)	0.95
Ara h 8	2.3 (0.01–21)	8.8 (0.22–21)	1.2 (0.01–13)	0.053
Ara h 9	0.01 (0.01–2.2)	0.01 (0.01–0.34)	0.01 (0.01–2.2)	0.19
Ara h 2 IgE-ab/IgE %	14.4 (5.2–50)	10.9 (5.2–15.4)	17.1 (5.8–50)	0.02
Ara h 1 IgE-ab/IgE %	7.4 (0–16.1)	3.2 (0–14.3)	7.8 (3.7–16.1)	0.028
Ara h 1 + 2 + 3 IgE-ab/IgE %	24.7 (5.9–68.4)	19.3 (5.9–30.2)	28.6 (13.9–68.4)	< 0.01
CD-sens	0.75 (0.24–20.5)	0.32 (0.24–5.5)	1.49 (0.44–20.5)	< 0.01
Omalizumab start dose (mg/4-week interval)	600 (150–1050)	675 (450–900)	450 (150–1050)	0.027
Omalizumab mg/kg/IgE	0.026 (0.017–0.056)	0.022 (0.017–0.024)	0.026 (0.018–0.056)	0.028

Numerical variables are presented as median (range). Coexisting atopic diseases are presented as prevalence (percentage).

Peanut challenge during omalizumab treatment

At peanut challenge, after 8–32 weeks with omalizumab at doses ranging from 100% to 400% (mean 147%) of the recommended dose, all patients ingested at least 840 mg of peanut protein. Mean dose given was 2352 mg, 15 of 23 patients (65%) completed the 2800 mg peanut protein (10 g of peanuts) final dose. Objective symptoms observed, among those completing the 2800 mg dose, were one case of localized urticaria and one case of rhinitis. Two patients developed unilateral conjunctivitis after poking an eye with a finger containing peanut residues.

Five of eight patients who did not reach the maximum dose (2800 mg) choose to stop due to subjective symptoms such as oral pruritus or mild self-resolving abdominal pain. The remaining three patients had objective symptoms: One patient got perioral erythema and two developed rhinitis. Also here we observed two cases of unilateral conjunctivitis after poking an eye with a finger containing peanut residues.

Mean Sampson score at the second challenge was 1.1 (0–2), significantly lower than the score of 3.8 at inclusion ($P = < 0.001$).

Among the 14 patients who went through two peanut challenges (at inclusion and during omalizumab treatment), the median ingested peanut dose was 50-fold increased (range 3.3–100-fold) at the second challenge (Table 3).

Baseline indicators and omalizumab dosing

Patients needing an elevated omalizumab dose (ED) ($n = 15$) had significantly higher CD-sens values at baseline compared to those with ND ($n = 8$); 1.49 (0.44–20.5) and 0.32 (0.24–5.5), respectively ($P = < 0.01$) (Fig. 2a).

No statistically significant differences were observed among the groups with regard to IgE-ab levels to peanut or peanut components, but total serum IgE levels were significantly lower in the ED group; 260 (81–860) kU/L compared to the ND group 660 (500–790) kU/L ($P = 0.0045$). However, the ratios between Ara h 2 IgE-ab/IgE and Ara h 1 IgE-ab/IgE as well as the sum of IgE-ab to peanut storage proteins Ara h 1, Ara h 2 and Ara h 3/IgE were significantly higher in the ED group compared to the ND group; $P = 0.02$, 0.028 and 0.008, respectively. The ND group had a

Table 2. Patient characteristics per study participant

ID	Age	Sex	IgE (kU/L)	Skin prick test Peanut (mm)	IgE-ab (kU _A /L)			Fraction Ara h 2 IgE-ab/IgE (%)	Asthma Yes/no	Eczema Yes/no	Other food allergy Yes/no
					Peanut	Ara h 2	Ara h 8				
1	19	F	670	11	350	170	0.01	25	No	No	No
2	12	F	690	13	91	66	21	10	No	Yes	No
3	18	F	860	15	200	66	13	8	Yes	Yes	Yes
4	15	M	260	9	79	38	1.7	15	Yes	Yes	No
5	16	F	410	12	48	24	6.2	6	Yes	No	No
6	18	M	680	11	325	185	0.49	30	Yes	No	Yes
7	16	F	690	11	260	66	15	10	Yes	No	No
8	16	F	540	11	86	78	5.5	14	Yes	No	Yes
9	16	F	710	9	165	75	0.98	11	Yes	No	Yes
10	18	F	790	9	168	102	0.22	13	Yes	Yes	Yes
11	18	F	160	10	41	30	0.1	19	Yes	No	Yes
12	17	F	500	9	35	26	2.3	5	Yes	Yes	Yes
13	14	M	130	9	52	16	0.01	12	Yes	No	Yes
14	18	F	540	10	250	61	12	11	Yes	No	Yes
15	17	M	470	10	200	55	2.4	12	Yes	No	Yes
16	17	F	340	5	85	58	5.1	17	No	No	Yes
17	15	M	630	8	220	97	18	15	Yes	No	No
18	14	M	230	13	48	26	6.6	11	Yes	No	No
19	17	F	81	8	34	32	0.75	40	Yes	Yes	Yes
20	17	F	140	8	32	24	1.2	17	Yes	No	Yes
21	13	M	440	12	290	220	4.0	50	Yes	No	No
22	16	F	250	13	75	50	0.68	20	Yes	No	Yes
23	17	F	100	11	30	20	0.61	20	Yes	No	Yes

median ratio of 11% of Ara h 2 IgE-ab/IgE compared to 17% in the ED group (Table 2, Fig. 2b).

No statistically significant differences were seen between the ND and ED groups in relation to age, gender, weight, atopic disease, SPT to peanut, severity score at food challenge or severity of most recent previous severe reaction to peanut (Tables 1–3).

Receiver operating characteristic-curves were calculated to evaluate which levels of CD-sens and fraction values of Ara h 2 IgE-ab/IgE that were indicative of a need of higher omalizumab doses (Fig. 2c and d). The area under the curve was 0.85 for CD-sens and 0.80 for the fraction Ara h 2 IgE-ab/IgE, with a cutoff level of > 0.59 for CD-sens and > 14.5% for Ara h 2 IgE-ab/IgE indicating the need of an elevated dose. At these cutoff levels, sensitivity for CD-sens was 80.0% and for Ara h 2 IgE-ab/IgE 66.7% and the specificity were 87.5% for both.

Discussion

This study shows that omalizumab effectively reduces allergen sensitivity to peanuts among severely peanut allergic adolescents. However, dosing omalizumab according to the recommendations for asthma might often not be enough. Low values of IgE and a high ratio for Ara h 2 IgE-ab/IgE as well as a high CD-sens seem to indicate need of an elevated omalizumab dose.

In the present study population, cutoff levels (calculated using ROC) indicating need of increased omalizumab were > 0.59 for CD-sens and > 14.5% for Ara h 2 IgE-ab/IgE.

In the present study, severely peanut allergic adolescents were treated with omalizumab to suppress their peanut allergy. Omalizumab was initially given at doses recommended for asthma, that is based on serum IgE level and body weight. Patients were treated for 8 weeks before treatment efficacy was evaluated with CD-sens. If CD-sens was still measurable, the omalizumab dose was increased and re-evaluated after another 8 week. The time frame consisting of 8 weeks of treatment (16 weeks is recommended for asthma) was used to rapidly find the optimal omalizumab dose. We have previously noticed, in pilot cases, that basophil allergen sensitivity decreases within 1–2 weeks during omalizumab treatment, due to the short life span of basophils [20]. In the present study, eight of 23 patients had a negative/favourable CD-sens to peanuts after 8 weeks, but the majority had not and continued treatment with an increased dose. However, one can speculate that this dose of omalizumab would have resulted in negative or favourable CD-sens in more patients if length of the treatment was longer, for example 16 weeks, especially in those patients with very low CD-sens values at week 8. It could also be argued that some of these patients already before

Table 3. Peanut challenge outcomes and omalizumab doses

ID	Anaphylaxis < 5 years	Symptoms at peanut challenge 1	Symptoms at anaphylaxis or challenge 1	Sampson score 1 or peanut intake	Sampson score challenge 1	Peanut protein dose (mg) challenge 1	Symptoms at challenge 2	Sampson score challenge 2	Peanut protein dose (mg) challenge 2	Increased peanut dose (factor) from challenge 1 to challenge 2	Omalizumab start dose mg/4 weeks	Omalizumab final dose mg/4 weeks	Final dose in % of start dose	Omalizumab 8-week cycles
Normal dose														
2	Y	a, ae, ap, f, epi x1		4			Perioral redness	1	868	n/a	450	→	100	1
7	Y	a, r, u, v, epi x1		4		28	op	1	2800	100	450 × 2	→	100	1
8	N	anx, ap, d, f, op, t, v		3		224	ap, op	1	1176	5	600	→	100	1
9	N	con, d, e, op, t, v, epi x1		4		280	op	1	2800	100	375 × 2	→	100	1
10	N	anx, ap, op, v, epi x1		4		140	op, f	1	1736	12.4	450 × 2	→	100	1
12	N	ap, d, h, m, op, t, u		4		56	op, r	2	2800	50	600	→	100	1
14	N	c, h, m, op, t		4		28	op, con	1	2800	100	600	→	100	1
17	N	ap, h, op, v, epi x1		4		56	con	1	2800	50	375 × 2	→	100	1
Elevated dose														
1	Y	ap, d, f, v, epi x2		3			None	0	2800	n/a	600	600 × 2	200	2
3	Y	a, h, p, u, epi x1		4			op	1	2800	n/a	525 × 2	600 × 2	114	2
5	Y	ae, d, u, v, epi x1		3			r	2	1876	n/a	600	450 × 2	150	2
11	N	a, c, con, d, e, op, r, t epi x2		4		28	None	0	2800	100	300	450	150	2
13	Y	a, e, u, v epi x1		4			u	1	2800	n/a	300	450	150	2
15	N	a, c, con, op, p, r, u epi x2		4		140	op	1	2800	20	600	450 × 2	150	2
16	Y	a, epi x1		4			op	1	2800	n/a	450	375 × 2	167	2
18	N	anx, con, op, t,		3		28	op, con	1	2240	80	300	450	150	2
20	Y	a, c, d, f, d-sat, epi x2		4			op	1	2800	n/a	300	450	150	2
21	Y	a, ap, u, v, epi x1		4			op, con	2	1148	n/a	600	450 × 2	150	2
22	N	ap, con, m, op, u, v		3		840	op	1	2800	3	300	450	150	2
23	N	anx, ap, op, r, v epi x2		4		126	op	1	2800	22	300	450	150	2
4	N	a, ap, d, d-sat, e, f, op, p, s, u, epi x3		4		280	op	1	2800	10	450	525 × 2	233	3
6	N	anx, ap, f, u, con, op, epi x1		4		28	op, r	2	1932	69	450 × 2	600 × 2	133	3
19	Y	a, t, u epi 1		4			op	2	1400	n/a	150	600	400	4

Y, yes; N, no; a, asthma; ae, angioedema; anx, marked anxiety or drowsiness; ap, abdominal pain; c, cough; con, conjunctivitis; d, dyspnoea; d-sat, desaturation < 91%; e, erythrodermia; f, feeling sick; h, hoarseness; m, muffled voice; op, oral pruritus; p, non-mucosal pruritus; r, rhinitis; s, stridor; t, throat tightness or difficulty swallowing; u, urticaria; v, vomiting; epi: epinephrine followed by number of doses; n/a: increased dose factor could not be calculated as only one peanut challenge was performed.

Table 4. CD-sens values to peanut

ID	CD-sens to peanut				
	Baseline	Week 8	Week 16	Week 24	Week 32
Normal dose					
2	0.57	0			
7	0.27	0			
8	5.50	0			
9	0.29	Favourable			
10	0.28	Favourable			
12	0.34	0			
14	0.52	Favourable			
17	0.24	Favourable			
Elevated dose					
1	1.57	0.12	0		
3	7.86	0.14	0		
5	1.49	0.14	0		
11	1.86	0.14	0		
13	0.52	0.12	Favourable		
15	20.5	0.5	Favourable		
16	1.47	0.14	0		
18	0.50	0.14	Favourable		
20	0.75	0.14	Favourable		
21	0.44	0.14	Favourable		
22	0.61	0.13	Favourable		
23	2.68	0.13	0		
4	1.77	0.15	0.16	0	
6	9.47	0.26	0.15	0.13*	
19	1.03	0.24	0.21	0.15	Favourable

Favourable = When basophils react only at the highest allergen concentration, a CD-sens value cannot be calculated, nor can the test be ruled as negative. In this study, a positive, non-calculable CD-sens value was regarded as favourable.

*Went on to peanut challenge despite slightly positive CD-sens.

reaching a favourable or negative CD-sens might have tolerated a higher amount of peanut, compared to baseline. However, previous studies show good correlations between CD-sens to peanut and clinical allergy to peanut, that is objective symptoms in DBPCFC [22, 24]. Finding objective markers of omalizumab treatment efficacy is important for optimal and individual dosage. Measurements of basophil allergen threshold sensitivity, for example CD-sens, have been shown to correlate significantly with the outcome of bronchial, nasal allergen titration challenge and oral challenges [22, 23, 29–31]. However, analysis of basophil reactivity might not always have the same correlation, as there is a distinct possibility to miss the optimal allergen concentration to allow a correct interpretation. It is of great importance not to confuse allergen sensitivity with reactivity. The correlation between CD-sens and clinical allergen sensitivity has been used, in previous studies, to identify omalizumab responders as well as to evaluate treatment efficacy after omalizumab dose adjustments [20, 21, 32].

In our study, we present data showing that patients who needed an elevated omalizumab dose (ED) had a higher CD-sens value at baseline compared to those where a normal dose of omalizumab (ND) was sufficient. Other studies evaluating the efficacy of omalizumab treatment in peanut allergy have also shown that some patients have insufficient treatment effect. In the light of our findings, we speculate that those non- or weak-responding patients might have had too low dose [14, 16]. Savage et al. used basophil histamine release (BHR) to follow omalizumab treatment efficacy. They found a tendency that patients with BHR < 20% tolerated more peanuts at peanut challenge, compared to patients with BHR level > 20%, even though the last mentioned group received a higher cumulative dose of omalizumab [16].

Among our 14 patients who performed peanut challenges both at inclusion and during omalizumab treatment, the ingested dose increased 50-fold ($P < 0.001$). All patients tolerated > 840 mg of peanut protein. It is likely that some of the patients would have tolerated more peanuts than the maximum dose (2800 mg) at the second challenge. Not only did the ingested peanut dose increase; the panorama of symptoms differed completely as illustrated by a significant decline in Sampson score. The most severe symptoms at challenge during concomitant omalizumab treatment were rhinitis and localized urticaria.

As shown in this study, as well as in a study of omalizumab treatment of severe milk allergy, CD-sens has the potential to reassure that the patient is given a sufficient dose of omalizumab to pass an oral food challenge [20].

As far as we know there are no previous publications presenting data indicating how to find an optimal omalizumab dose for treating peanut allergy. In this study we analysed baseline data in relation to the need of elevated omalizumab dose, looking for indicators that could predict a need of an elevated dose. One such marker is baseline CD-sens, which was significantly higher in the ED group, compared to ND group. In addition, we saw significantly lower levels of IgE, and higher ratios of Ara h 2 IgE-ab/IgE and higher ratios of the sum of Ara h 1, Ara h 2 and Ara h 3 IgE-ab/IgE in the ED group compared to the ND group. Thus, a high ratio could indicate the need of a higher dose of omalizumab than recommended for asthma. In the present study, cutoff levels for the CD-sens value and the ratio for Ara h 2 IgE-ab/IgE, indicating a need of higher omalizumab dose, were calculated through ROC-curves, with good sensitivity and specificity. Given the small study size, these results must be interpreted with caution. While both CD-sens and the ratio of Ara h 2 IgE-ab/IgE may be good indicators of the need of an

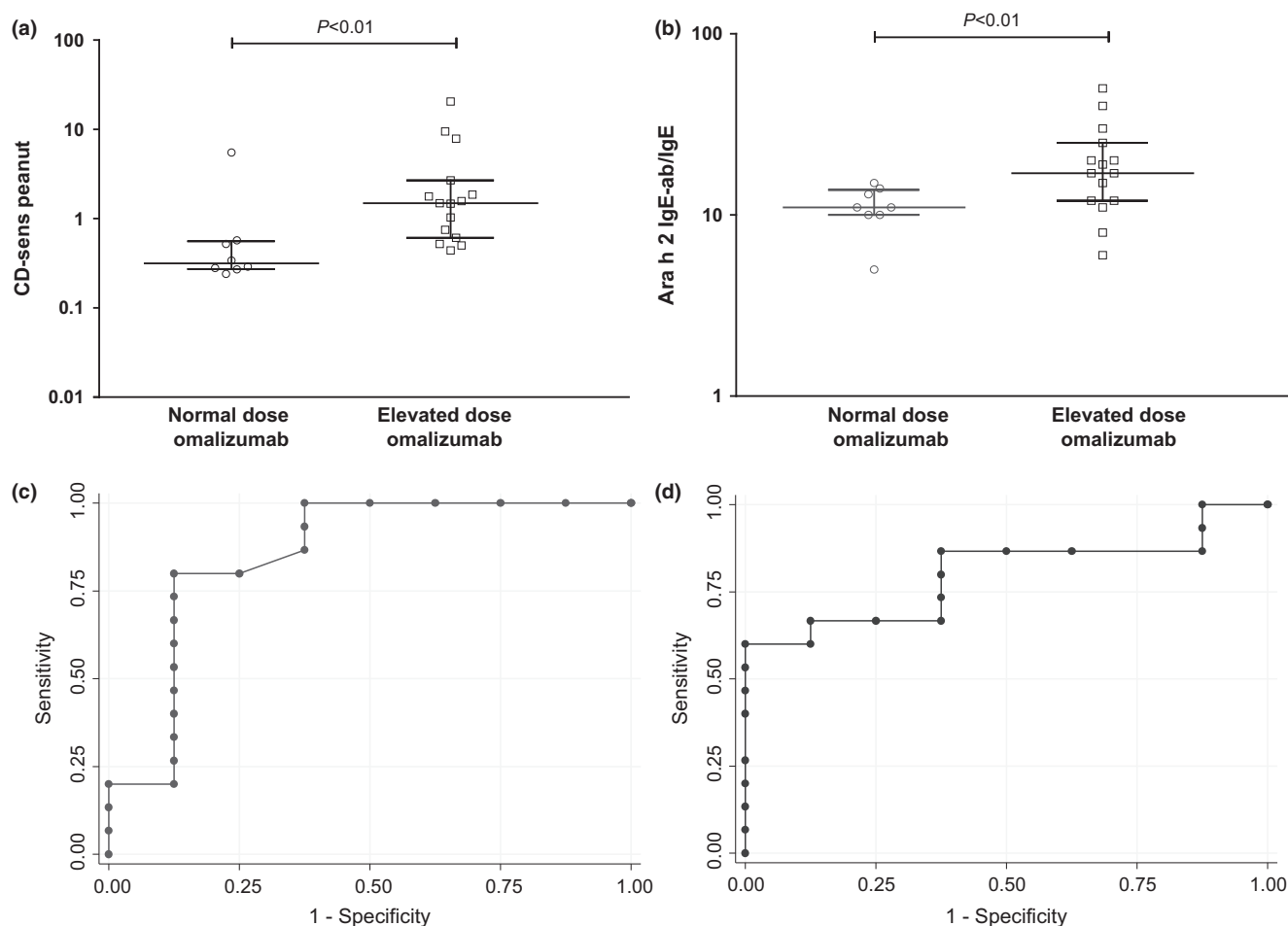


Fig. 2. Baseline values of CD-sens (a) and Ara h 2 IgE-ab/IgE (b) in relation to normal or elevated omalizumab dose. Receiver operating characteristic curves (ROC) for CD-sens (c) and Ara h 2 IgE-ab/IgE (d) as indicators of a need for an increased omalizumab dose.

elevated omalizumab dose, only CD-sens may be used to follow omalizumab treatment efficacy.

One weakness of this study is the low number of participants and one-armed study design. Therefore, our study has to be considered as hypothesis generating. Further, to fully assess the ability of the CD-sens test to correctly indicate the effectiveness of omalizumab treatment, it would have been very interesting, albeit ethically questionable, to let patients with a measurable CD-sens value go through additional peanut challenges every 8th week. However, it has previously been shown that basophil allergen threshold sensitivity to peanut seem to correlate with the outcome of oral peanut challenge [22, 23], but these findings need further corroboration and are not validated when an intervention such as omalizumab has been provided. Also the favourable CD-sens-value, known to the clinician and to some extent by the participants, may have affected evaluation of subjective symptoms. The major strength is that all study subjects did either have a convincing medical

history of a recent peanut-induced anaphylaxis or had severe objective allergic symptoms at peanut challenge at inclusion. This makes this study unique when it comes to exclusively studying the severe, and thus most important, phenotype. Although we have shown in this study that omalizumab treatment can effectively suppress peanut allergy, it must be stated that it is an expensive treatment. Given the limited resources most communities have with regard to healthcare expenses, the cost-effectiveness of this therapy therefore needs to be addressed. We would like to see studies that address cost-benefit aspects of treating food allergies with Xolair. Also the effects from this intervention is most likely not persistent and future studies should address effectiveness of long-term treatment and combined therapies such as omalizumab in combination with specific immunotherapy.

In conclusion, we have for the first time shown that individually dosed omalizumab, leading to a negative or favourable CD-sens is an effective intervention for

severe peanut allergy. Given the small sample size and one-armed study design, the results from this trial should be looked upon as hypothesis generating and need confirmation in larger placebo-controlled studies. Using the fraction size of IgE-ab to storage proteins, for example Ara h 2/IgE, as well as CD-sens to peanut, it may be possible to predict the need of an elevated omalizumab dose. In addition, CD-sens may also be used to measure and follow omalizumab treatment efficacy.

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Conflict of interest

This study was conducted without any involvement or financial support from Novartis. Josef Brandström has received lecture fees from Novartis. Caroline Nilsson has received lecture fees from Novartis and Thermo Fisher. The other authors have no conflict of interests to declare.

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