

Original Article

Clinical Efficacy and Immunological Effects of Omalizumab in Allergic Bronchopulmonary Aspergillosis

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What is already known about this topic? Allergic bronchopulmonary aspergillosis (ABPA) often presents with persistently uncontrolled asthma despite the use of corticosteroids and antifungal therapy. Patients are generally excluded from therapy with omalizumab, a humanized anti-IgE antibody, due to their very high serum IgE levels.

What does this article add to our knowledge? We show both clinical and immunological efficacy of omalizumab in patients with ABPA, for the first time, in a randomized, placebo-controlled trial, confirming previous case studies and uncontrolled case series.

How does this study impact current management guidelines? Omalizumab can be prescribed, as a safe and effective therapy, to patients with ABPA with serum total IgE levels exceeding current dosing recommendations.

BACKGROUND: Allergic bronchopulmonary aspergillosis (ABPA) often presents with persistently uncontrolled asthma despite the use of corticosteroids and antifungal therapy. Omalizumab is a humanized anti-IgE monoclonal antibody currently used to treat severe asthma.

OBJECTIVE: The aim was to assess the clinical and immunological effects of omalizumab in ABPA in a randomized, placebo-controlled trial.

METHODS: Patients with chronic ABPA were randomized to 4-month treatment with omalizumab (750 mg monthly) or placebo followed by a 3-month washout period in a cross-over design. The main endpoint was number of exacerbations. Other clinical endpoints included lung function, exhaled nitric oxide (FeNO), quality of life and symptoms. *In vitro* basophil activation to *Aspergillus fumigatus* extract and basophil FcεR1 and surface-bound IgE levels were assessed by flow cytometry.

RESULTS: Thirteen patients were recruited with mean total IgE 2314 ± 2125 IU/mL. Exacerbations occurred less frequently during the active treatment phase compared with the placebo period (2 vs 12 events, $P = .048$). Mean FeNO decreased from 30.5 to 17.1 ppb during omalizumab treatment ($P = .03$). Basophil sensitivity to *A. fumigatus* and surface-bound IgE and FcεR1 levels decreased significantly after omalizumab but not after placebo.

CONCLUSION: Omalizumab can be used safely to treat ABPA, despite high serum IgE levels. Clinical improvement was accompanied by decreased basophil reactivity to *A. fumigatus* and FcεR1 and surface-bound IgE levels. © 2015 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2015;■:■-■)

Key words: Omalizumab; Allergic bronchopulmonary aspergillosis; Basophils; Clinical trial

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Allergic bronchopulmonary aspergillosis (ABPA) manifests as deteriorating asthma, elevated total and *Aspergillus*-specific serum IgE levels, and immediate skin prick test (SPT) reactivity to *Aspergillus fumigatus* antigens.¹⁻³ Additionally, ABPA complicates asthma in approximately 2% of cases.³ Standard pharmacotherapy for ABPA involves corticosteroid and antifungal therapy,^{4,5} but, despite these medications, ABPA may progress to a chronic stage of corticosteroid-dependent asthma complicated by bronchiectasis.⁶ To manage severe asthma symptoms and avoid recurrence of acute ABPA, patients may require continuous oral corticosteroid treatment in addition to maximal inhaled asthma therapies, indicating a requirement for additional asthma treatments.⁷

Omalizumab, a member of the biological class of medications, is a humanized monoclonal IgG₁ antibody to IgE that binds to free serum IgE, which leads to downregulation of the cell-surface high-affinity IgE receptor (FcεR1) on basophils and mast cells.⁸ Omalizumab is effective in the treatment of allergic asthma in adults and children,^{9,10} which reduces exacerbations, symptoms,

Abbreviations used

ABPA- Allergic bronchopulmonary aspergillosis
 AP- Allophycocyanin
 BAT- Basophil activation test
 CF- Cystic fibrosis
 FEV₁- Forced expiratory volume in 1 second
 FeNO- Fractional exhaled nitric oxide
 FITC- Flourescein isothiocyanate
 fMLP- Formyl-methionyl-leucyl-phenylalanine
 PE- Phycoerythrin
 PBMC- Peripheral blood mononuclear cells
 QOL- Quality of life
 SPT- Skin prick test

and the need for other medications in asthma control.¹¹ Dose recommendations for omalizumab are derived to reduce free serum IgE to less than 50 ng/mL (20.8 IU/mL),¹² so dosing recommendations are based on a patient's baseline total IgE and weight (kg) (0.016 mg kg/IU [IgE/mL]) to a maximum of 750 mg monthly. Patients with ABPA generally exceed the current dosing parameters of omalizumab due to their often very high serum IgE levels. Despite this, there have been several case reports¹³⁻¹⁶ and uncontrolled case series¹⁷⁻²⁰ that describe ABPA treatment with omalizumab. Although these studies show a potential role for omalizumab in ABPA treatment, a conclusive evaluation of efficacy by a placebo-controlled randomized trial has not yet been reported.

The effects of omalizumab can be monitored immunologically. The basophil activation test (BAT) is an *in vitro* cellular assay for basophil surface CD63 expression as a marker of cell degranulation.²¹ Using the BAT, a dose-response curve of basophil activation by allergen can be used to determine the basophil allergen threshold sensitivity (basophil sensitivity).²² Basophil sensitivity has been shown to correlate well with clinical tests for allergen sensitivity including SPT and allergen provocation and has been used to monitor the efficacy of therapies including allergen-specific immunotherapy and omalizumab.²³⁻²⁵ As patients with ABPA have serum IgE against a known specific allergen, *in vitro* basophil studies can also be used to monitor the effects of omalizumab in this disease, independent of clinical results. Basophil reactivity could provide a new clinically relevant indicator for the efficacy of omalizumab in patients with high serum IgE levels.

We conducted a randomized controlled cross-over study to assess the clinical efficacy of omalizumab in chronic ABPA and to determine the underlying immune mechanisms involved by the analysis of basophil reactivity.

METHODS**Subjects and study design**

Subjects more than 18 years of age who met the diagnostic criteria for ABPA, as defined by Rosenberg and Patterson,¹ were recruited from a tertiary hospital clinic. Participants were included if they had poorly controlled symptoms of ABPA with cough, sputum production, wheeze, and dyspnea despite treatment with maximal doses of inhaled combined corticosteroid and long-acting β -agonist therapy and regular oral corticosteroid if required. All patients met the additional inclusion criteria of a previous diagnosis of asthma, an elevated serum total IgE > 400 IU/mL, the presence of proximal bronchiectasis on chest high-resolution computerized tomography

scan, sensitization to *A. fumigatus* antigens with a positive SPT > 3 mm, and the presence of *Aspergillus*-specific serum IgE.^{26,27} Total and allergen-specific IgE were measured with the UniCAP system (Pharmacia, Sweden). SPT was performed using standardized reagents (Hollister-Steir, Spokane, Wash). Subjects were excluded if they had commenced antifungal therapy in the previous 8 weeks or had required an escalation of corticosteroid treatment during that time. Cystic fibrosis (CF), pregnancy, and lactation were also the exclusion criteria. Maintenance asthma and ABPA treatments were held constant for the study duration. The study was a randomized, double-blind, placebo-controlled, cross-over trial (Figure 1) with 2 treatment phases of 4-month duration. Baseline characteristics were recorded at study entry (time point zero). Patients were then randomized into 2 phases: treatment with omalizumab, or placebo. Omalizumab at a dose of 375 mg, based on the dose chart, or normal saline (placebo) was administered subcutaneously every 2 weeks with an intervening 3-month washout period. The sequence of treatments was randomized by the hospital pharmacy that prepared the injections. Clinicians, laboratory research staff, and the nurse responsible for administration of the injections remained blinded to the patient's treatment phase. Ethics approval was provided by the Alfred Hospital Ethics Committee (273/08). All subjects provided written informed consent. The study was registered in the Australian New Zealand Clinical Trials Registry No. ACTRN12613000278730.

Clinical measurements

The primary endpoint was the number of exacerbations during the treatment or the placebo phase. It was estimated that a clinically significant reduction in exacerbations of 40% would be measured with 90% power by estimating a standard deviation of 1.5 (50% of anticipated mean) with 12 participants. This was inflated to 14 to account for dropouts. All patients were reviewed monthly throughout the study. The patients were advised to report any increase in symptoms between visits to the clinical investigators (AG, JD), which was then followed by a clinical assessment and spirometry. An exacerbation was defined as worsening of respiratory symptoms (increased shortness of breath, cough, wheeze, and/or sputum) that necessitated treatment with prednisolone to a maximum of 50 mg per day, which reduced over 5 to 7 days, and antimicrobial therapy where indicated. Secondary endpoints included changes in spirometry, performed according to the American Thoracic Society recommendations (ATS)²⁸; fractional exhaled nitric oxide (FeNO), performed according to the joint ATS/The European Respiratory Society guidelines²⁹ using a *Medisoft Hypair* device (Medisoft, Sorinnes, Belgium); asthma-specific quality of life (QOL) score³⁰; and asthma symptoms that were recorded by the following standardized questions at every clinic visit: (1) "In the past week, how many puffs of reliever medication have you used on average each day?" (2) "In the past week, how many nights have you been woken by asthma?" At 0, 4, 7, and 11 months (before and after each treatment phase), measurements made included spirometry, FeNO, QOL score, and asthma symptoms, and blood samples were taken for immunological assessment of basophil activation and IgE receptor levels. At the monthly visits, participants were clinically evaluated, symptoms recorded, and spirometry was performed. Regular asthma treatment, including maintenance oral corticosteroid, was kept unchanged throughout the study. In accordance with Australian guidelines for the prescription of omalizumab, patients were provided with an EpiPen autoinjector (Mylan Specialty LP, Basking Ridge, NJ) to be used in the event of anaphylaxis. Patients

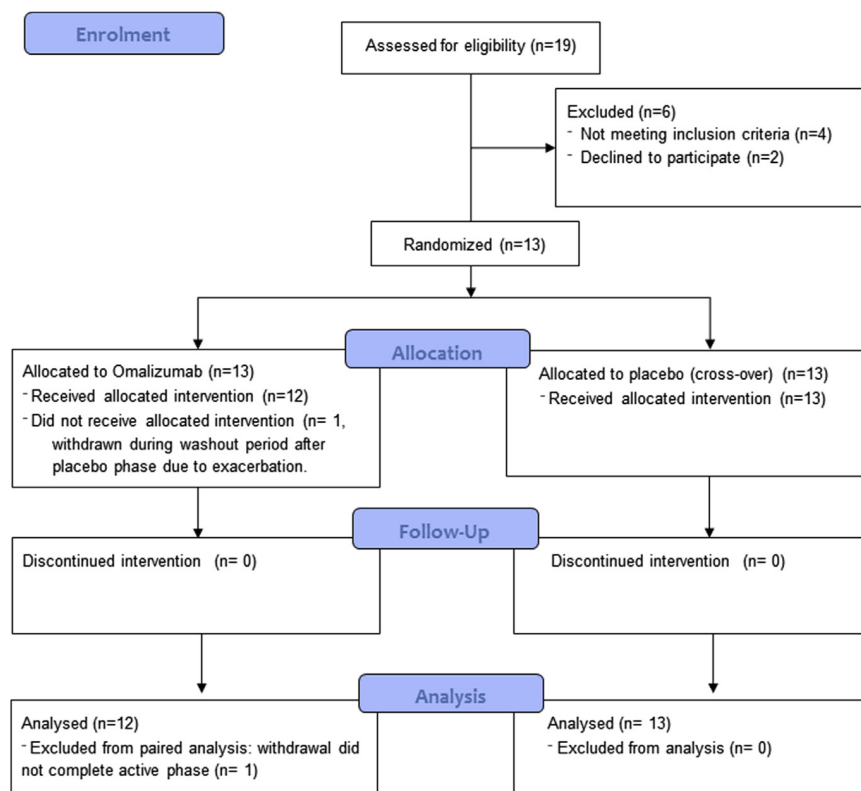


FIGURE 1. CONSORT flow diagram.

were withdrawn from the trial if they suffered an exacerbation that necessitated more than 10 days of escalated oral corticosteroid therapy or required hospitalization.

Immunological measurements

BAT, FcεR1, and surface-bound IgE. Immunological data were collected at 0, 4, 7, and 11 months. Basophil activation was analyzed as described previously,³¹ in both 100 μL heparinized blood and ficoll-isolated peripheral blood mononuclear cells (PBMC) (5×10^6 mL⁻¹). Analysis within PBMC was performed to exclude any plasma-derived factors that may interfere with basophil activation. Specifically, high levels of (antigen-specific) IgE bound to omalizumab may accumulate as this complex formation increases the half-life of IgE in plasma.³² Concentrations ranging from 50 pg/mL to 50 μg/mL *A. fumigatus* extract (Greer, Lenoir, NC) were used to determine a dose-response curve of cell activation. Positive controls were N-formyl-methionyl-leucyl-phenylalanine (fMLP; Sigma-Aldrich, Munich, Germany) and rabbit antihuman IgE (DAKO, Carpinteria, Calif). The negative control was 100 μL stimulation buffer alone. Cells were then stained with anti-IgE-FITC, anti-CD63-PE (Invitrogen Life Technologies, Carlsbad, Calif), and anti-CD203c (basophil-specific marker)-APC (Miltenyi Biotec, Bergisch Gladbach, Germany).

FcεR1 cell-surface expression and surface-bound IgE levels were examined separately on whole blood and PBMC basophils, respectively. Cells were stimulated for 20 min with stimulation buffer³² to induce IL-3-mediated upregulation of CD203c for more accurate detection of the basophil population. They were then stained with anti-IgE-FITC or anti-FcεR1-PE (eBioscience, San Diego, Calif) and anti-CD203c-APC.

After staining, the cells were washed, resuspended in wash buffer containing 7-amino-actinomycin D (7AAD; BD Pharmingen, San Diego, Calif), and stored on ice until flow cytometer acquisition (FACSCalibur; BD Biosciences, San Jose, Calif) with the CellQuest software (BD, Mountain View, Calif) using consistent instrument settings for each patient throughout treatment. Between 500 and 1000 basophils were acquired for each sample and then analyzed with the TreeStar FlowJo analysis software (Tree Star, San Carlos, Calif). Corresponding isotype control antibodies were used to determine thresholds of detection. The basophil population was identified as IgE, CD203c double positive cells with CD63 as the activation marker, presented as a percentage of the entire basophil population.

Calculation of basophil reactivity and sensitivity. Basophil reactivity represents the highest percentage of basophil activation achieved within the dose-response curve after stimulation with increasing concentrations of *A. fumigatus* extract. Basophil allergen threshold sensitivity (CD-sens) was determined as described previously²² and used to compare basophil sensitivity before and after therapy and between omalizumab and placebo treatment. Basophil sensitivity was calculated as the inverted value of the allergen concentration (ng/mL) required for 50% maximal basophil reactivity (EC50) multiplied by 100 ($1/\text{EC50} \times 100$). In cases where a plateau of the dose-response curve was not reached, the maximal basophil reactivity at baseline (pretreatment) was used to calculate sensitivity. If 50% maximal basophil reactivity was not achieved within the concentration range tested, sensitivity was described as 0.002 (the lowest value measurable within the allergen concentration range).

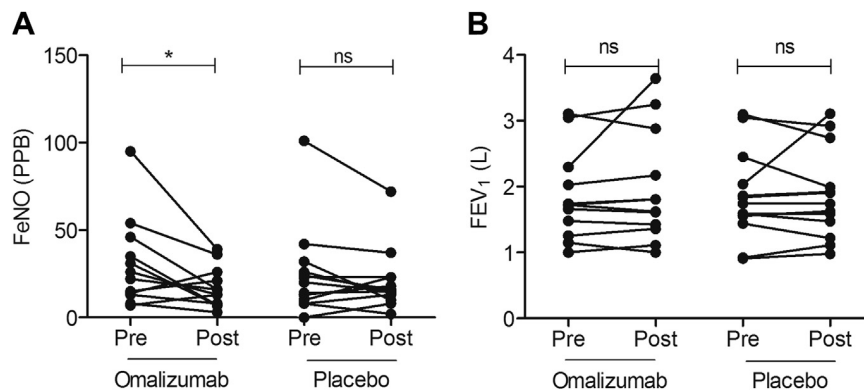


FIGURE 2. FeNO and lung function. There was a significant reduction of FeNO with omalizumab compared with baseline ($P = .03$, paired t -test). $*P < .05$. FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second.

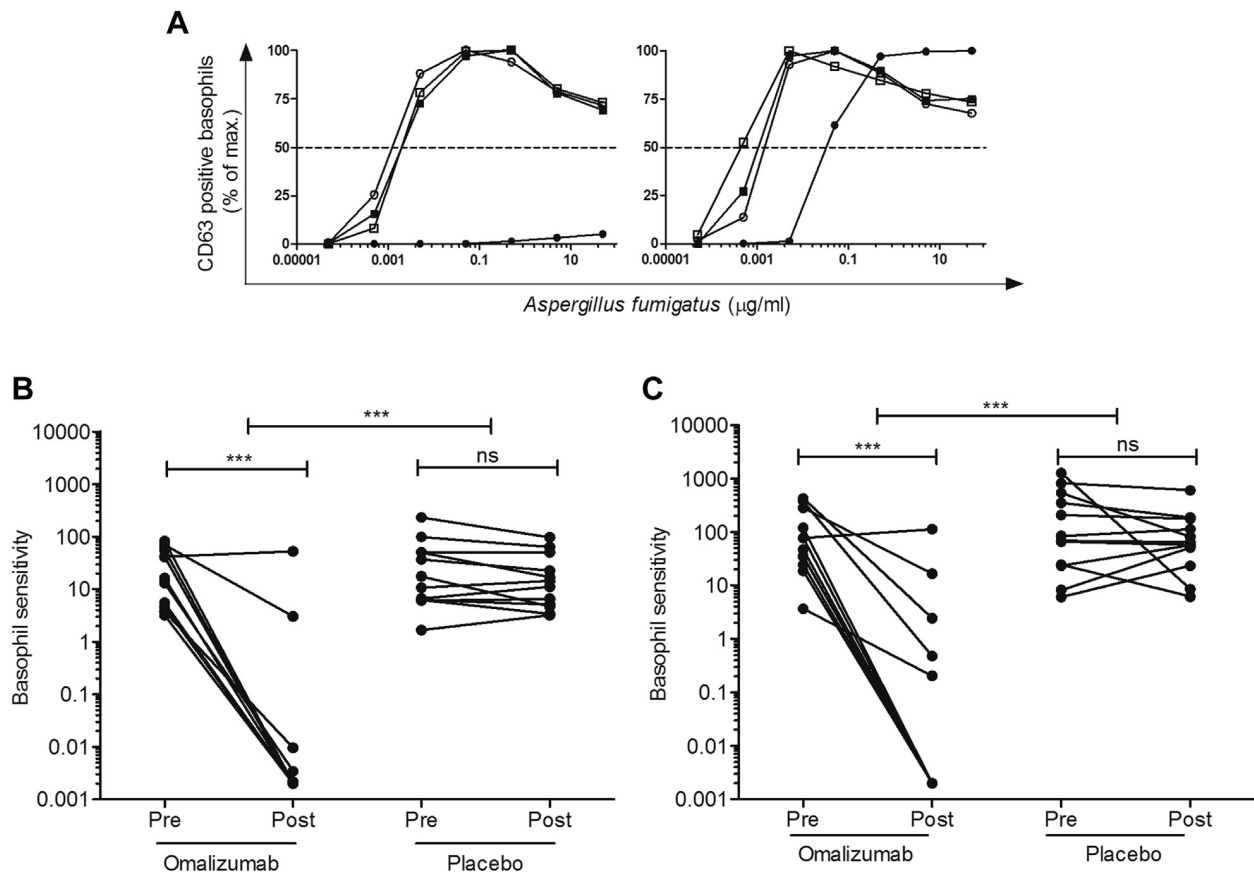


FIGURE 3. Basophil sensitivity. Representative graphs showing the dose-response curve of basophil activation (shown as a percentage of maximal activation) in response to *Aspergillus fumigatus* allergen stimulation before (○) and after (●) omalizumab compared with before (□) and after (■) placebo. (a) The dashed line indicates EC50, used to calculate the sensitivity. Basophil sensitivity decreased significantly after omalizumab treatment but not placebo in both whole blood (b) and PBMC (c) derived basophils, as calculated with the Wilcoxon-signed rank test on log-transformed data. Differences between phases were calculated with the Wilcoxon signed rank test. $***P < .001$.

Statistical analysis

For analysis of clinical data, all patients were included on an intention-to-treat basis. Clinical and immunological parameters were assessed for normality. The change from pre-to posttreatment values

was compared using a paired t -test, where appropriate, or the Wilcoxon matched-pairs signed rank test if assumptions for normality could not be achieved by data transformation. A P -value of $< .05$ was considered statistically significant. All statistical analyses were

TABLE I. Baseline characteristics of all 13 participants at the initial study visit

Mean age (y)	59 (26-69)
Female (no. of subjects)	9
Weight (kg)	73.2 (52-97.3)
Total IgE (IU/mL)	2314 (346-7860)
(<700 , >700 -1000, >1000)	(3, 1, 9)
Skin prick test to <i>Aspergillus</i> (mm)	7.42 (3-11)
<i>Aspergillus</i> -specific IgE (IU/mL)	50.0 (2.9-100)
Blood eosinophils ($\times 10^9 \text{ L}^{-1}$)	0.50 (0.1-2.7)
FEV ₁ (L)	1.93 (1.00-3.20)
FEV ₁ (% predicted)	73.2 (47-136)
($>80\%$, 60% to 80% , $<60\%$)	(3, 5, 5)
FEV ₁ /FVC % (FER)	59.4 (41-85)
FeNO (ppb)	29.92 (7-95)
(<25 , 25 -50, >50)	(7, 5, 1)
Inhaled corticosteroid (no. of subjects)	13
Mean daily inhaled corticosteroid dosage (Beclomethasone dipropionate equivalent, μg)	1100
Maintenance long acting beta agonist (no. of subjects)	12
Maintenance prednisolone (no. of subjects)	6
Mean oral prednisolone daily dose (mg/d) n = 6	8.4
Antifungal therapy (no. of subjects)	4

FER, forced expiratory ratio; FEV₁, forced expiratory volume in 1 second; FeNO, fractional exhaled nitric oxide; FVC, forced vital capacity.

Data are presented as mean (range). Number of patients in categories are shown for total IgE, FEV₁ (% predicted) and FeNO.

performed with the Graphpad Prism 5 software (Graphpad, San Diego, Calif).

RESULTS

Clinical outcomes

Thirteen participants were included in the study. Table I outlines the baseline characteristics and medication requirements at study entry. A significant decrease in the number of exacerbations was observed with omalizumab. Two patients experienced one exacerbation each requiring treatment with prednisolone during the omalizumab phase compared with a total of 12 exacerbations occurring in 6 patients in the placebo phase ($P = .048$, Wilcoxon matched-pairs signed rank test) (Table II). Each patient who experienced an exacerbation in the placebo phase experienced a reduction of exacerbations in the omalizumab phase.

Secondary outcomes are presented in Table III and Figure 2. There was a significant reduction in FeNO ($P = .03$, paired t -test) (Figure 2, *a*) and night waking ($P = .012$, Wilcoxon matched-pairs signed rank test) in the treatment phase but not during the placebo period ($P = .97$ and $P = .68$, respectively); however, when comparing the treatment and placebo phases, the difference did not reach statistical significance for either parameter.

Immunological outcomes

Representative dose-response curves are depicted in Figure 3(*a*). Basophil sensitivity to *A. fumigatus* decreased significantly after the omalizumab but not placebo phase. This decrease was observed in both whole blood and isolated PBMC derived basophils (Figure 3, *b* and *c*, respectively). In addition to the basophil sensitivity levels, the maximal basophil reactivity

achieved also decreased significantly in the omalizumab phase (Table IV). Basophil activation by positive control anti-IgE did not change significantly during omalizumab or placebo treatment, whereas fMLP-induced basophil activation increased in the omalizumab ($P = .02$) but not placebo phase; however, the difference between phases was not statistically significant. The percentage of basophils within the PBMC population did not change after treatment (Table IV). Basophil surface Fc ϵ R1 and IgE levels decreased significantly in the omalizumab phase compared with the placebo phase ($P = .002$ and $P = .0005$, respectively) (Figure 4).

An indication of the serum IgE threshold for omalizumab treatment may have been found in the single patient who did not show decreased basophil sensitivity to *A. fumigatus* or decreased basophil Fc ϵ R1 levels on treatment. The total serum IgE level of this patient before treatment was 7860 IU/mL, which constituted the highest IgE level of all participants by over 2800 IU/mL.

To assess for day-to-day instrument (FACSCalibur) and material (fluorochrome-labeled antibodies) variations, a posttrial screen of Fc ϵ R1 and IgE levels of 2 patients was performed on cryopreserved PBMC from each time point. No difference was found between changes in Fc ϵ R1 and IgE levels measured during the trial and posttrial (data not shown).

Clinical and immunological baseline values did not differ statistically between the placebo and omalizumab phase, which indicated no carry-over effect.

Safety

There were no significant adverse outcomes observed during the study period related to omalizumab use; specifically there were no episodes of anaphylaxis. One patient was withdrawn from the trial during the washout period due to an exacerbation that required hospitalization and at study completion was found to have received placebo in the preceding trial phase. An additional patient was excluded from the immunological data as his or her basophils failed to activate to an IgE-cross-linking stimulus (including *A. fumigatus* antigen and anti-IgE antibody), termed nonreleaser basophils.³³

DISCUSSION

To our knowledge, this is the first study that evaluates the effects of omalizumab on ABPA in a randomized, placebo-controlled trial. This study provides evidence that omalizumab is an effective therapy for patients with chronic ABPA, which results in a decreased number of exacerbations during the active treatment phase compared to placebo and a decline of a well-accepted inflammatory marker of the lower airways (FeNO). These findings suggest that the immunological mechanisms underlying exacerbations in ABPA can be effectively reduced with omalizumab, with the potential to prevent disease progression and/or development of bronchiectasis.

A limitation to this study is the cohort size. A power analysis demonstrated that 12 participants were required to detect a clinically significant reduction in exacerbations. Although a statistically significant decrease in FeNO was detected in the omalizumab phase, this decrease was not significant when compared with the placebo phase. In a larger cohort, FeNO may serve as a marker of response to treatment with omalizumab in subjects with ABPA, as is the case in asthma. FeNO is a Th2-driven inflammatory biomarker generated by the enzyme inducible nitric oxide synthase, which is induced by IL-13.³⁴ A recent large randomized

TABLE II. Exacerbation frequency

Subject	Placebo FEV ₁ (% pred)	Omalizumab FEV ₁ (% pred)	Exacerbations placebo phase	Exacerbations omalizumab phase
1	85%	70%	0	0
2	136%	132%	0	0
3	69%	65%	2	1
4	77%	76%	0	0
5	61%	53%	1	0
6	40%	50%	0	0
7	80%	75%	0	0
8	60%	61%	2	1
9	43%	47%	0	0
10	66%	73%	0	0
11	96%	96%	1	0
12	74%	50%	3	0
13	NA	67%	3	Withdrawn

FEV₁, forced expiratory volume in 1 second; NA, not available.

Exacerbation frequency of each individual patient and percentage predicted FEV₁ at the beginning of each phase.

TABLE III. Secondary outcomes

	Omalizumab		Placebo		P-value
	Time 0	4 mo	Time 0	4 mo	
FEV ₁ (L) ^a	1.84 ± 0.66	1.98 ± 0.85	1.96 ± 0.80	1.87 ± 0.68	.16
% Predicted FEV ₁ ^b	67.5 ± 25	72 ± 22.5	69 ± 22	69 ± 24.5	.12
FeNO (ppb) ^a	30.5 ± 25.12	17.1 ± 11.5	24.7 ± 25.6	20.5 ± 17.6	.14
Quality of life (QOL) ^b	1.0 ± 1.18	0.88 ± 0.90	1.06 ± 1.09	1.21 ± 0.65	.58
β ₂ agonist use ^a	2.1 ± 1.5	1.5 ± 1.3	2.6 ± 1.83	2.2 ± 2.2	.71
Night waking due to asthma ^b	2.0 ± 2.5	0.50 ± 1	2.5 ± 2.75	1.5 ± 2.5	.16

FEV₁, forced expiratory volume in 1 second; FeNO, fractional exhaled nitric oxide.

Data are presented as mean ± SD (a) or median ± IQR (b). P-value represents the difference between the omalizumab and placebo phases calculated with the paired *t*-test (a) or the Wilcoxon matched-pairs signed rank test (b).

TABLE IV. Basophil maximal reactivity

	Omalizumab		Placebo		P-value
	Time 0	4 mo	Time 0	4 mo	
Whole blood ^a	90.4 ± 12.2	35.5 ± 63.1	90 ± 8.4	89.9 ± 10.7	.002
PBMC ^b	83.1 ± 21.7	30.3 ± 62.1	81.6 ± 17.5	78.8 ± 19.7	.001
Anti-IgE ^c	44.6 ± 26.3	55.6 ± 22.3	42.6 ± 15.1	40.2 ± 22.5	.40
fMLP ^d	39.2 ± 23.6	48.6 ± 17.8	37.0 ± 20.3	41.6 ± 19.8	.15
% basophils ^e	2.2 ± 0.8	2.3 ± 1.3	2.2 ± 1.2	2.2 ± 1.1	.69

PBMC, peripheral blood mononuclear cells; fMLP, formyl-methionyl-leucyl-phenylalanine.

The change in maximal basophil activation levels achieved in both whole blood (a) and isolated PBMC (b) assays in response to *Aspergillus fumigatus* extract. Data are presented as median ± IQR. P-values shown indicate the difference between the omalizumab and placebo phases, calculated with the Wilcoxon matched-pairs signed rank test. Activation levels induced by anti-IgE (c) and fMLP (d) and the percentage of basophils (e) within the PBMC population are presented as mean ± SD. Differences between the omalizumab and placebo phases were calculated with the paired *t*-test.

placebo-controlled multicenter study that evaluated omalizumab response in subjects with moderate-to-severe asthma demonstrated that high baseline levels of FeNO was a predictor of response to omalizumab therapy in these subjects. The omalizumab group demonstrated a 46% reduction in exacerbations versus the placebo group in the high FeNO subgroup, compared with a 31% reduction in the low FeNO subgroup after 48 weeks of treatment using a cutoff level of FeNO > 24 ppb.³⁵

Our results confirm earlier case reports and case series that suggested the clinical efficacy of omalizumab in treating ABPA.^{16,36,37} In a recent retrospective review of uncontrolled

cases that reported the efficacy of omalizumab in patients suffering from ABPA without CF, similar results to this study were obtained, where patients experienced fewer exacerbations but with no consistent improvement in FEV₁ and QOL scores.¹⁶ As this study excluded patients with CF, where ABPA is considered common,^{38–40} these results should not be extrapolated to this condition. Notably, a recent summary of previous reports that assessed the efficacy of omalizumab in children with ABPA and CF demonstrated that omalizumab appeared to be associated with improvements in lung function, reduction in symptoms and recurrent exacerbations, and decreased use of systemic

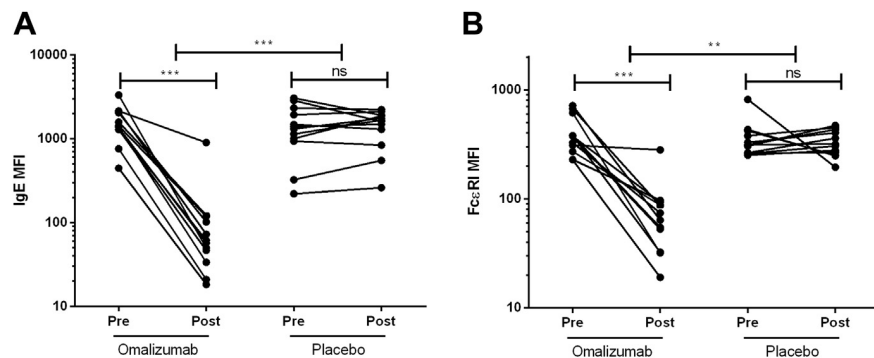


FIGURE 4. Basophil surface FcεR1 and IgE. Basophil surface-bound IgE (a) and FcεR1 expression (b) before and after the omalizumab and placebo phase. Values represent the mean fluorescence intensity (MFI) of each marker for individual patients in each phase. Differences between phases were calculated with the Wilcoxon signed rank test. ** $P < .01$; *** $P < .001$.

corticosteroids.³⁵ The conflicting results in lung function with omalizumab across the case reports and reviews are not surprising given the heterogeneity of subjects, variability of severity and duration of disease and variable presence of chronic irreversible airways damage. In addition, despite clinical improvement, pulmonary function test results seldom change in patients with allergic asthma after treatment with omalizumab.⁴¹ Changes in exacerbations and corticosteroid sparing effects are better measures of efficacy given that lung function is not an ideal parameter for this purpose in ABPA. We were not able to define clear changes in corticosteroid doses in our study because only 6 subjects were taking oral corticosteroids. Recent studies suggest the importance of diagnosing *Aspergillus* and fungal sensitization in patients with severe asthma due to typically worse asthma outcomes in this group⁴² and poor response to antifungal therapy.⁴³ Our findings encourage trials of omalizumab in allergic fungal airway disease, including severe asthma with fungal sensitization.

This study also provides substantial *in vitro* correlates, with a significant decrease in basophil sensitivity to the *A. fumigatus* antigen and in maximal basophil reactivity after omalizumab, but not placebo, as well as a significant reduction in basophil FcεR1 expression and surface IgE levels. As 10 of the 13 patients had a total baseline serum IgE level well in excess of the maximum threshold for omalizumab administration (700 IU/mL), our results provide direct evidence for the effective use of omalizumab in patients with ABPA who currently do not meet the inclusion criteria for this treatment.

Previous studies have shown a significant reduction in basophil surface FcεR1 and IgE with omalizumab therapy.^{8,23} Studies in severe asthma indicate that the mast cell presence and FcεR1 density in both central and peripheral airways are major features of severe asthma.⁴⁴ The relative contribution of mast cells and basophils to allergic inflammatory processes has been demonstrated in a study using intranasal cat allergen challenges where basophil, but not mast cell responses, correlated with the effectiveness of omalizumab treatment.²⁵ The immunological data of our study clearly show that omalizumab was effective in decreasing both basophil sensitivity and maximal reactivity to *A. fumigatus* as well as basophil surface FcεR1 and IgE levels in patients with total serum IgE levels of up to 5000 IU/mL and *Aspergillus*-specific IgE levels of up to 78 IU/mL. These results indicate that a lack of clinical improvement in some patients was not due to suboptimal dosing of omalizumab. This is the first

study to show the mechanistic efficacy of omalizumab in patients with serum IgE levels of up to 5000 IU/mL, over 7 times the current recommended dosing threshold.

Omalizumab is an effective treatment for patients with allergic asthma⁹; however, current dosing recommendations exclude many patients with ABPA due to high baseline levels of serum IgE.^{9,10} The dosing recommendations were generated from early clinical studies demonstrating that reducing free IgE to less than 50 ng/mL was associated with better clinical outcomes for allergic asthma.¹² Our study provides evidence for effective and safe omalizumab treatment in patients with ABPA with total serum IgE beyond current dosing recommendations.

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