OMALIZUMAB IN SEVERE ASTHMA: EFFECT ON ORAL CORTICOSTEROID EXPOSURE AND REMODELING.

A randomized open-label parallel study.

Short title: Omalizumab in severe asthma: effect on oral corticosteroid exposure and remodelling

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Acknowledgements.

We thank Anna Garrit for her technical assistance in electron microscopy laboratory (Department of Morphological Sciences- UAB), Natàlia Martínez, nurse at the Respiratory Service (Corporació Parc Taulí-UAB) and Dr. Vicente Marco-Molina (Servei d'Anatomía Patològica, Hospital Quirón (Barcelona).

Key points:

- The study shows the OC-sparing effect and restorative capacity on bronchial epithelium of a biological treatment.
- Contrary to previously held beliefs, it shows that bronchial remodeling in patients with severe asthma is reversible with biologic therapy despite a reduction in the dose of oral corticosteroids.
- It allows reinterpretation of the histological changes found, so that the thickening of the basement membrane appears to be a protective reaction of the epithelium to exert the barrier function lost with the damage of the disease.
- It reinforces the current idea of the importance of the bronchial epithelium in the pathophysiology of asthma (asthma as "epithelial driven disease").

Abstract word count: 341

Body text word count: 4573

Abstract

Introduction: Data on the clinical efficacy and remodeling of omalizumab therapy in patients on oral corticosteroids (OC) are limited.

Objective: The purpose of the study is to show that in patients with corticosteroid dependent asthma, omalizumab is a corticosteroid-sparing therapy able to inhibit airways remodeling and to reduce disease burden (lung function impairment, exacerbations).

Methods: CHOC is a randomised open-label study evaluating the addition of omalizumab to the standard of care in patients with severe asthma receiving oral corticosteroids. The primary endpoint was represented by the change in OC monthly dose by the end of treatment and secondary endpoints included spirometry changes, airway inflammation (FeNO), number of exacerbations and airways remodelling assessed by bronchial biopsies studied by transmission electron microscopy. As safety variable, adverse effects were collected.

Results: Efficacy was assessed for 16 patients in the omalizumab group and 13 in the control group. The final cumulative mean monthly OC doses were 34.7 and 217 mg for the omalizumab and control group respectively; the mean difference between groups adjusted for baseline was -148.1 [95% CI: -243.6, -52.5] (p=0.004). OC withdrawal: 75% vs. 7.7%; p=0.001).
Omalizumab provided: a slowing of FEV1 loss (70 mL vs 260 mL), a significant decrease in FENO values and a reduction in the annual relative risk of clinically significant exacerbations of 54%. The treatment was well tolerated. The morphological study showed a significant decrease in basement membrane thickness in the omalizumab group (6.7 vs. 4.6 µm) compared to controls (6.9 vs. 7. µm) (mean difference between groups adjusted for baseline = -2.4 [95% CI: -3.7, -1.2] p<0.001), as well as a decrease in intercellular spaces (1.18 vs. 0.62 µm and 1.21 vs. 1.20 µm]; p=0.011) respectively). A qualitative improvement was also observed in the treated group.

Conclusions: Omalizumab showed a marked OC-sparing capacity and was associated with an improvement in clinical management that correlated with bronchial epithelial repair. In OC-dependent asthma, reversibility of remodeling is possible; the concepts that basement membrane enlargement is detrimental and that chronic airway obstruction is systematically irreversible are outdated (EudraCT: 2009-010914-31).

1.- INTRODUCTION.

Over the last 15 years, a new drug family has emerged for the treatment of severe asthma: the monoclonal antibodies (mAb). Initially recommended as the last step for treatment of severe uncontrolled asthma, recent guidelines now place mAb administration in front of that of oral corticosteroids.

The first mAb to be marketed was omalizumab, a humanized murine anti-IgE for allergic asthma (1,2). The pivotal studies were published at the beginning of this century (3-6). Since then, several other mAbs have been marketed (7). The requirements for registration have evolved and become more strict: regulatory agencies now expect companies to demonstrate clinical benefits (decrease in exacerbations and hospital admissions, improvement of pulmonary function testing, inhaled corticosteroid sparing effect) not only in moderate/severe asthma but in oral corticosteroid (OC) dependent patients as well (8-10).

To date, a vast amount of papers have been published on omalizumab and have shown it to be well tolerated and clinically useful in the management of moderate/severe allergic asthma (3-6). In spite of this, the clinical information regarding certain aspects of the drug's activity is limited and the information on its performance in some specific situations which have in fact been assessed in studies describing the development of more recent mAbs is absent. Moreover, the information about the potential effects of mAbs on remodeling is scarce or absent.

To fill this gap, we designed a study to evaluate the **C**linical and **H**istological impact of treatment with **O**malizumab in severe allergic oral **C**orticosteroid dependent allergic asthma patients (the CHOC study).

2.- MATERIAL AND METHODS.

The hypothesis to be tested was the following: Omalizumab, an anti-IgE recombinant humanized monoclonal antibody, is a well-tolerated drug that aids the management of allergic corticosteroid-dependent asthma patients. Its strong points are: a) It allows a reduction in OC intake; b) It improves clinical management of patients by decreasing exacerbations and stabilizing pulmonary function; c) It reverses histopathological changes in the bronchial wall, and also the chronic inflammation.

2.1 Population: Patients were recruited from the asthma out-patient clinics of the Pulmonary Services at two hospitals (Hospital Parc Taulí and Hospital Clínico Universitario de Santiago de Compostela, Spain). Briefly, the study included adult allergic corticodependent asthma patients. The inclusion/exclusion criteria are described in the online resource 1. Briefly, the population studied comprised adult allergic OC-dependent asthma patients eligible for omalizumab treatment who were not receiving specific immunotherapy.

2.2 Outcomes: *Primary efficacy outcome*: a) Change in OCS monthly dose by the end of treatment; *Secondary efficacy outcomes*: a) Spirometry changes; b) Airway inflammation (fraction exhaled of nitric oxide [FeNO]; c) Number of exacerbations; d) Reversibility of the histological changes in the bronchial mucosa. *Safety outcomes*: a) Adverse events related to drug treatment.

2.3 Type of study: Phase IV, open-label randomized parallel study (EudraCT: 2009-010914-31) with two groups treated with best standard care receiving OC: the omalizumab group receiving omalizumab as add-on therapy and the control group not receiving omalizumab.

Although the study had an open design, the pathologist and the clinicians were blinded to the clinical and pathological findings respectively.

***2.4 Stages of the study**:* The study comprises three stages (online resource 2).

2.4.1 Stabilization period: The stabilization period was planned to last at least three months. During this period patients received the best standard care for asthma according to GINA (Salmeterol /Fluticasone 50/500 Acuhaler® bid, montelukast 10 mg/once a day, OC and salbutamol on request). At the beginning of this period, patients receiving prednisolone were switched to 6-methyl-prednisolone (6-MP); 4 mg of 6-MP were considered equivalent to 5 mg of prednisolone. OC doses were progressively tapered (2 mg/day of 6-MP every two weeks) until a decrease of 10% or more in the forced expiratory volume in one second (FEV_1) was observed. The daily dose of 6-MP was then increased by 2 mg every two weeks until the FEV1 returned to its previous levels and the process was repeated. At the third failed attempt to reduce the OC dose, we considered that the minimum dose required had been attained. A written informed consent was obtained.

2.4.2 Run-in period: A 4-week period, before randomization after the patient had agreed to participate in the trial.

2.4.3 Follow-up/Treatment period: Patients were randomized to receive or not receive a subcutaneous dose of omalizumab. Omalizumab dose was based on body weight and initial serum immunoglobulin E (IgE) (approximately equal to 0.016mg/Kg x body weight (in kg) x IgE (in International Units/mL) per 4 weeks for a 12-month period. During the treatment period, the oral corticosteroid decrease protocol followed was the same than during the stabilization period (6-MP was progressively tapered (2 mg/day every two weeks) until FEV_1 fell by 10% or more; the 6-MP dose was then increased to the previous dose until the FEV1 returned to its previous levels). The procedure was repeated throughout the follow-up.

2.5 Randomization: Patients were assigned to their group according to a randomization list linked to a treatment arm. Patients were allocated to one or other arm according to a ratio 1:1.

2.6 Study protocol:

2.6.1 Definition of exacerbations: Exacerbations were classified into three groups. Mild exacerbations were those that required occasional (< 3 days) increase of OC and/or antibiotics. Moderate exacerbations included increase of OC intake (≥ twice the baseline dose) during ≥ 3 days and/or antibiotics + unscheduled ambulatory visit. Severe exacerbations included increased OC intake (≥ twice the baseline dose) ≥ 3 days and/or antibiotics + hospital admission.

2.6.2 Measurements:

At the beginning and end of the study, the following tests were performed in each patient: skin prick test, spirometry, FeNO measurement, blood analysis (including IgE), bronchoscopy with bronchial biopsies for morphological study (transmission electronic microscopy [TEM]).

After six months: blood analysis including IgE.

Monthly: spirometry, FeNO measurement, daily OC dose, monthly accumulated OC dose, recording of exacerbations and side-effects.

Technical details of every test can be found in the online resource 3 and 4.

Morphological study of bronchial biopsies (Transmission electron microscopy-TEM).

The processing of biopsies for electron microscopy is described in the online resources 5-8. We studied the degree of the epithelial damage focusing on the thickness of the basement membrane, the intercellular space of the epithelium and characteristics of the epithelium and cilia following standardized methods (11, 12). The measurements were performed twice by the same EM expert, in two different time periods separated by a minimum of three months.

2.7 Sample size calculation. The sample size for this study was 28 randomized patients in each of two treatment groups. The sample size was calculated to achieve a power greater than 80% using a two-sided z-test at a significance level of 5% and based on the primary efficacy endpoint –percentual change from baseline in monthly oral corticosteroids at the end of follow-up–, expecting to find decreases of 50% (standard deviation [SD]=50) in the omalizumab group and 10% (SD=50) in the control group.

An interim efficacy analysis was planned after 50% of recruitment, that was oversaw by an independent data monitoring committee. Test boundaries were determined using the Pocock spending function (see Table 1s in online resource 9).

2.8 Statistical analysis.

Between-group differences in changes (end minus baseline) of continuous outcomes (both clinical and morphological) were analysed by means of linear regression models adjusting for basal measures. End-study measures from the morphological study were analysed by means of logistic regression models for dichotomous outcomes and proportional odds ordinal logistic regression models for ordered outcomes, adjusting for basal measures. Longitudinal analysis of all clinical measurements collected throughout the months of follow-up was performed by means of generalised linear mixed effects models, including interaction term between study group and follow-up time in all models allowing the groups' changes to differ over time. Annual exacerbation rate ratios (total and by severity) were estimated using negative binomial generalised linear models.

R version 4.2.0 was used for all analyses (13). The mixed model function of the **GLMMadaptive** package (14) was used to fit the mixed-effects models; the vglm function of the **VGAM** package (15) was used to fit ordinal logistic models; the glm.nb function of the

MASS package (16) was used to fit negative binomial models; and results were visualized using the **ggplot2** package (17).

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

2.9 Institutional review board approval.

The study was approved by the Institutional Review Board of both hospitals. Written informed consent was obtained from every patient. The study was registered with EudraCT number 2009-010914-31. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

3.- RESULTS.

Initially, 31 patients were randomized, of which two were excluded. The study was stopped for efficacy, with 16 patients in the omalizumab group and 13 patients in the control group, and proceeded to the analysis of the rest of the data (figure 1).

3.1 **Clinical results**. At entry, both groups exhibited similar demographic and clinical characteristics (Table 1).

3.1.1 Oral corticosteroid use. At the end of follow-up, mean monthly accumulated OC doses were 34.7 mg (SD=81.6) and 217 mg (SD=186) for the omalizumab and the control group, respectively. These figures corresponded to mean percentual changes from baseline of 79% less (SD=36) in the omalizumab group and 61% more (SD=191) in the control group (figure 2a) and the mean difference between groups adjusted for basal was -148.1 [95% CI: -243.6, -52.5] (p=0.004). Mean absolute differences were -163.8 mg (SD=102) and -8.8 mg (SD=196) for the omalizumab and the control group, respectively (figure 2b); when adjusted for basal measures,

mean difference between groups was -170.5 [95% CI: -264.5, -76.5] (p<0.001). After removing extreme values from the control group as sensitivity analysis, the adjusted mean differences were -96.1 [-139.4, -52.9] (p<0.001) in percentual changes and -132.7 [-195.9, -69.5] (p<0.001) in absolute changes. Twelve (75%) patients in the omalizumab group and only 1 (7.7%) patient in the control group withdrawn OC treatment (p = 0.001).

3.1.2 Pulmonary function tests. Regarding pulmonary function, at the end of follow-up the control group lost 260 mL (SD=580) while the omalizumab group only lost 70 mL (SD=480) from baseline (mean difference between groups adjusted for basal = 226.2 [95% CI: -185.7, 638.1], p=0.268). In percent scale, FEV_1 decreased 7.9% (SD=19.1) and 4.3% (18.3) in the control and the omalizumab group, respectively (mean difference between groups adjusted for basal = 2.79 [95% CI: -10.3, 15.9], p=0.662) (figures 2c-d).

3.1.3 Airway inflammation. Significant difference between groups was found in change from baseline in airway inflammation measured by FeNO values (mean difference between groups adjusted for basal = -17.3 [95% CI: -25.6, -8.94], p<0.001), with a reduction of 14.8 ppb (SD=23.5) in the omalizumab group while the control group experienced an increment of 4.3 ppb (SD=14) (figure 2e). Note that mean final FeNO value in the omalizumab group was 17.7 parts per billion (ppb) (normal value).

3.1.4 Immunoglobulin E changes. Significant difference between groups was also found in change from baseline in IgE values (mean difference between groups adjusted for basal = 425.8 [95% CI: 199.5, 652.0], p<0.001), observing a mean decrement of 20.8 IU/mL (SD=268) in the control group and a mean increment of 355 IU/mL (SD=320) in the omalizumab group (Figure 2F), equivalent to a mean ratio of 3.8 compared to baseline. Additional data are given in the online resource 10 (figures 1s, 2s and 3s).

3.1.5 Exacerbations. The estimated annual rates of clinically significant exacerbations were 1.12 (95% CI = 0.67, 1.88) in the omalizumab group and 2.46 (95% CI = 1.6, 3.78) in the control group figure 2g). Thus, compared with the control group, the annual exacerbation rate ratio was 0.46 (95% CI = 0.23, 0.89). The annual relative risk reduction was 54%. By severity, the annual rate ratio for mild exacerbations was 0.88 (95% CI = 0.37, 2.1); and for moderate exacerbations was 0.31 (95% CI = 0.09, 0.97). No severe exacerbations were observed in the omalizumab group during follow-up. Mean duration of the exacerbations was 13 days (SD=14.8) in the omalizumab group and 31 days (SD=33.6) in the control group (p=0.093). Additional data are given in the online resource 10 (figures 4s and 5s).

3.1.6 Longitudinal trends of the clinical outcomes by study groups. The longitudinal analysis showed that lung function, airways inflammation and OC cumulative monthly dose showed constantly more favourable trends in omalizumab group (figure 3) (interaction term p<0.001). As expected, IgE levels increased over time for the omalizumab group but no for the control group (see also figure 6s in the online resource 10).

3.2 Morphological study.

No statistically significant differences were observed between the two groups at entry in any of the parameters studied.

3.2.1 Thickness of the basement membrane (BM). Figure 5 shows the thickening of BM. The omalizumab group showed a marked decrease in BM thickness, while controls presented a slight increase (mean difference between groups adjusted for basal = -2.4 [95% CI: -3.7, -1.2], p<0.001). The decrease in BM thickness occurred in 13 out of 14 patients (92.9%) of the omalizumab group and in only 3 out of 10 (30%) of the control group (figure 4).

3.2.2 Intercellular space. Similar to thickness of the BM, the omalizumab group showed a marked decrease in intercellular spaces, and controls remained stable (mean difference between

groups adjusted for basal = -0.56 [95% CI: -0.98, -0.14], p=0.011). In the omalizumab group all patients presented reductions in the intercellular space compared to only 5 out of 10 controls (figures 4 and 5).

3.2.3 Damage to the epithelium and cilia description. We observed considerable detachment of the bronchial epithelium(fig ure 5). In the first biopsy (at entry), patients in the omalizumab group showed more severe damage in the epithelium (two patients showed a total loss of the columnar epithelium) than patients in the control group, although there were no statistically significant differences between groups (online resource 10, figures 7s). Statistically significant differences were found between groups at the end of the study adjusting for basal damage (p=0.0499). Additionally, a higher percentage of improvers during the follow-up in the omalizumab group (64.3%) than the control group (20.0%) was observed (online resource 10, figures 8s). The two patients of the omalizumab group who showed at entry a total loss of the columnar epithelium showed reappearance of the columnar cells although some detachment was still present.

Regarding the extent (focal or extensive) of the damage to the epithelium, the percentage of patients in the omalizumab group with focal involvement increased from 35.7% to 50% at the end of the study, while in the control group the percentage of patients showing extensive involvement notably increased from 70% to 90% (online resource 10, figures 9s). Nevertheless, adjusting for basal measures, no statistically significant difference between groups at the end of the study was observed (p=0.064). Finally, regarding the qualitative evaluation of the changes observed in the cilia, the percentage of patients in the omalizumab group with no absence or focal absence increased from 35.7% to 71.4% at the end of the study, while in the control group these figures decreased from 50% to 40%. Adjusting for basal measures, a

statistically significant difference between groups at the end of the study was observed (p=0.038).

In the initial and final biopsies, we observed some ultrastructural abnormalities such as axoneme bubble, presence of only one singlet and supernumerary singlets, presence of compound cilia, and ciliary oedema and ciliary disorientation. Abnormalities present in the first biopsy persisted in the second biopsy, independently of the group. When ciliogenesis occurred in the second biopsy (mainly in the omalizumab group), ultrastructural abnormalities were rare, except for the presence of some ciliary disorientation (Figure 10s).

3.3 Side effects. Regarding side effects, the treatment was well tolerated and there some side effects reported in the online resource 11. Only arthromyalgia, that persisted at the end of the treatment could be considered a drug-related side effect. None patient abandoned the treatment.

4.- DISCUSSION

The purpose of our study was to elucidate the clinical and histological benefits of a one-year treatment with omalizumab in the most severe population of asthma patients, namely those who are OC-dependent. Omalizumab has demonstrated its ICS-corticosteroid sparing capacity, but there were no randomized clinical trials designed to show its OCS-sparing capacity. The Cochrane review (18) concluded that omalizumab treatment was associated with a significant probability of reducing inhaled corticosteroid doses or of withdrawing them completely. The mean dose reduction was -118 μg equivalent of beclomethasone dipropionate. In the subgroup of patients who received oral steroids, it was not clear whether this benefit occurred. In a subgroup analysis of an open-label parallel group study, Siergiejko et al (19) found that at week 32, 62.7% of patients in the treated group were able to stop or reduce OCs compared to 30.4% of controls. The real-life study eXpeRience (20) found a relative reduction of 50% at month 24, and APEX (a UK retrospective study) found a 34% reduction in the mean total quantity of OCs prescribed per year and complete cessation of OC use after 12 months in 48% of patients (21). In a two-year observational prospective study (22), we found that omalizumab allowed OC withdrawal in 74.2% of patients. It is normally considered that real-life studies obtain better results than randomized clinical trials, but CHOC presented results of the same order as the best real-life studies.

One of the key parameters used by regulatory agencies for registering a mAb is its capacity to decrease the rate and the severity of exacerbations. The INNOVATE study (23) showed that the clinically significant exacerbation rate (the primary efficacy variable), adjusted for an observed imbalance in exacerbation history, was 0.68 with omalizumab and 0.91 with placebo (26% reduction) during the 28-week treatment phase. Without adjustment, a similar magnitude of effect was seen (a reduction of 19%), although it did not reach statistical significance. In our

study, the annual exacerbation rate ratio showed a 54% reduction of total exacerbations. Regarding severe exacerbations, the INNOVATE (23) study showed a 50% annualized reduction. In our study none of the omalizumab patients presented severe exacerbations during follow-up. In addition, in our study the duration of the exacerbation was halved. To summarize, our data show that omalizumab helped to reduce exacerbations, especially the most severe ones. Two real-life studies involving OC-dependent patients (eXpeRience registry (20) with 28.1% of patients receiving OC and in the Xpertise trial (24) with 46%) showed a decrease in exacerbations of above 80%

In addition to exacerbations, some studies have measured the emergency room visits or hospitalizations. The INNOVATE (23) study, in which OC-dependent patients accounted for 22% of the sample and 78% were on inhaled corticosteroid, reported an annualized 44% reduction in emergency room visits. In a real-life study, Molimard et al (25) found a 65% reduction in annual emergency visits following omalizumab therapy. In Germany, in a population of 280 patients of whom 46% were OC-dependent, Korn et al (24) reported an 82% reduction in exacerbations and a 78% reduction in hospitalizations. In our study, emergency visits were addressed indirectly. Exacerbations were defined as severe if they required hospital admission. The absence of severe exacerbations in the omalizumab group indirectly reflects the absence of hospitalizations despite the decrease or withdrawal of OCs.

Another purpose of a biological treatment is to improve or minimize the loss of lung function. In OC-dependent patients, the main point is to demonstrate this at the same time as the reduction in OC use. The Normansell Cochrane review (18) included two studies with 732 participants and showed a significant difference in the mean improvement of FEV_1 compared to baseline in moderate/severe asthma (67.29 mL). The eXpeRience trial (20) showed an improvement in FEV_1 of 8.7% after two years. No information is available on pulmonary function testing

changes in OC-dependent patients. We observed FEV_1 losses of 70 mL in the treated group and 260 mL in the control group. We regard these differences as being clinically relevant. After one year of treatment, omalizumab was able to slow down the loss of lung function in the most severe group of patients although the loss continued to be higher than expected in a healthy population. There are two possible explanations for this finding: the limited follow-up (maybe a longer treatment period might bring down the loss of FEV1 to within normal limits) or the limited effect on the thickness of the smooth muscular layer.

The changes in airway inflammation are represented by FeNO values. FENO reflects the activity of IL13 which is released by Th2 lymphocytes as well as innate lymphoid cells. Significant changes were observed at the end of the follow-up. Moreover, in the omalizumab group, the final mean FeNO value was within normal limits (below 25 ppb, the value specified by GINA for diagnosis of a T2 asthma). Thus, omalizumab treatment clearly contributed to downregulating the bronchial inflammation, despite the decrease in OC intake. In the control group, the increased FENO values reflect the activity of the Th2 cascade and/or the persistence of the stimulus of alarmins whose release does not diminish because the integrity of the bronchial ciliated epithelium does not improve.

Finally, as expected, there was a marked increase in blood IgE concentration, reflecting the treatment with the anti-IgE blocker (22). Regarding safety, no relevant side effects related to treatment occurred that limited the treatment with omalizumab.

The histological study of the bronchial biopsies was performed by electronic microscopy. (26, 27). In the last two decades, the histopathological changes that occur in asthma have attracted attention. Initially, inflammation was identified as the main pathophysiological cause of chronicity of the disease. Later, attention has focused on the disruption of bronchial epithelium

(26), and the loss of cilia and thickening of the basement membrane of the epithelium (27) (especially the reticular layer).

The histological improvement observed in our study was dramatic. At entry, a marked denudation of the bronchial mucosa was observed, with loss of cilia, increased intercellular space, and cell loss. The BM was also markedly thickened, but treatment with omalizumab reduced its thickness. BM thickening has traditionally been considered to be irreversible once it occurs. Riccio et al (28) in a study without a control group already suggested that some degree of reversibility could occur as a result of omalizumab treatment. Our study is the first to stablish that this benefit occurs after omalizumab in comparison with a control group. The BM enlargement probably develops as a protective mechanism, with the allergen or other impurities on one side and the antigen presenting cells on the other. The thicker the BM, the less likely the interaction between external noxae and subepithelial cells. Considering that the barrier function of the bronchial epithelium is markedly impaired, the thickening of the BM may contribute to regulating the deterioration of asthma. The bronchial cells are normally next to each other and there is no intercellular space, and desmosomes help to maintain the structure and the barrier function of the epithelium. One of the first disorders that occurs in cells is the loss of cilia and subsequently, the loss of their function as cleaners of the mucosa, meaning that pollutants, allergens, and virus are able to remain in the airway. The appearance of intercellular space is a sign that precedes the absolute denudation of the mucosa. In some patients the cell loss is total; the BM comes into direct contact with the airway lumen and is exposed to pollutants, virus and allergens. At this point, the functions of the bronchial epithelium break down. Although chronic treatment with OC has shown a certain reparative effect in some patients, the benefits observed in most of the patients receiving omalizumab were notably greater. These morphological changes should help to improve asthma control, decrease exacerbations, and achieve other

clinical benefits, in some cases allowing the withdrawal of the drug (29). Thus, our results challenge the belief that chronic remodeling is irreversible, that the thickening of the BM is harmful, and that chronic airway obstruction, when present, is irreversible (30).

There are several possible explanations for these morphological changes. “In vitro” studies have shown that several cells, especially smooth muscle cells, have receptors for IgE. This causes hyperplasia and hypertrophy of the smooth muscle layer (31). The presence of IgE has also been shown to favour the depositing of collagen fibers and extracellular matrix (32). The blockade of the binding of IgE to its receptor prevents or postpones these deposits (32). The remodeling caused by the presence of IgE can also be prevented by omalizumab (33). This has a synergistic effect with the blockade of acetylcholine which also favours the deposition of extracellular matrix (34). It should be noted that the influence of IgE in the remodeling process occurs in the absence of allergens. The blockade of IgE postpones these changes and the histological changes are manifested in a decrease in the thickness of the bronchial wall (35) or, in some cases, a complete reversibility of the airway obstruction (36). Finally, the repair of the bronchial epithelium contributes to a decrease in the release of alarmins, thus limiting their harmful effect.

The most important limitation of the study is the lack of placebo in the control group. We could not obtain an oily solution similar to omalizumab and thus we would have had to use a placebo that would have had a very different appearance. Since the nurses collaborating in the study in charge of treatment administration would have easily differentiated the placebo from omalizumab, and the treatment was administered at the hospital day unit (where patients on biologics are usually treated) the possibility that patients would have identified the treatment/placebo was very high. Therefore, we thought it was better to plan the study as a parallel randomized but open-label study. To minimize the influence of the lack of placebo in

the interpretation of the results, the pathologist, as well as the clinicians, were blinded to the clinical and pathological findings, respectively.

5.- Conclusions. In summary, CHOC fills in some of the gaps in the literature regarding the benefits omalizumab can offer in OC-dependent allergic asthma patients. It is the first randomized study to show the clinical benefits of omalizumab including the slowing down the loss of respiratory function despite the OC decrease. Moreover, it is also the first study to show the histological abnormalities that occur in severe allergic asthma patients with chronic OC treatment, and the capacity of omalizumab to facilitate the repair of the bronchial epithelium, given that some of the patients returned to normality. It thus prompts us to rethink the interpretation of remodeling changes, and to abandon the widespread idea that chronic remodeling is irreversible. The histological recovery of the bronchial mucosa can help to explain the improvement in clinical management of the patients (35) and also the feasibility of withdrawing omalizumab in certain patients (29). This would be in line with the current view on the importance of the bronchial epithelium in the pathophysiology of severe asthma (37). The results of CHOC study invalidate the current interpretations of the data. The idea that BM thickening is detrimental can be considered outdated and erroneous. This process occurs in order to replace the barrier or protective function of the bronchial epithelium, which is damaged in asthma patients. Taken together, the results lead us to conclude that omalizumab is a disease-modifying drug. Finally, the concept of remission has recently been introduced. Future research may clarify whether the changes observed in CHOC can help explain the clinical remission achieved by some patients (38).

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TABLES

Table 1. Baseline data for omalizumab group and control group

	Omalizumab group N=16	**Control group N=13**
Age, mean (SD)	47.2 (14.0)	46.5 (13.9)
Women, n (%)	11 (68.8%)	8 (61.5%)
FEV_1 (%), mean (SD)	78.4 (20.2)	79.8 (12.3)
FeNO (ppb), median (P25, P75)	18.0 (14.0, 38.0)	18.5 (14.5, 36.5)
IgE (IU/mL), median (P25, P75)	96.5 (71.2, 169)	145 (80.0, 332)
Monthly OC dose (mg), median (P25, P75)	150 (120, 248)	240 (90.0, 300)
Daily OC dose (mg), median (P25, P75)	4.5 (4.0, 6.5)	6.0 (4.0, 8.0)

FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; IgE, Immunoglobulin E; OC, oral corticosteroid; P25, 25th percentile; P75, 75th percentile; SD, standard deviation.

FIGURE LEGENDS

Figure 1. Study flowchart.

Figure 2. Changes from baseline in clinical outcomes at the end of follow-up and annual exacerbation rate by study group and severity.

FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; IgE, Immunoglobulin E; OC, oral corticosteroid

Figure 3. Longitudinal trends of clinical outcomes by study group

FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; IgE, Immunoglobulin E; OC, oral corticosteroid

Figure 4. Transmission electron microscopy outcomes

Figure 5. Improvement in epithelial damage in the omalizumab group.

Note the changes in the bronchial biopsy before and after one year of omalizumab treatment. Figure 5-a shows the thickening of the BM and the absence of bronchial epithelial cells. Figure 5-b shows the return to normal, disappearance of intercellular spaces and reappearance of cilia.

Figure 5-c shows the initial biopsy from another patient, showing the disappearance of cilia, the appearance of intercellular spaces and denudation of cells. Figure 5-d (12 months after omalizumab treatment) shows a picture of normal ciliated epithelium with palisade cells and reappearance of cilia.

Figure 1.

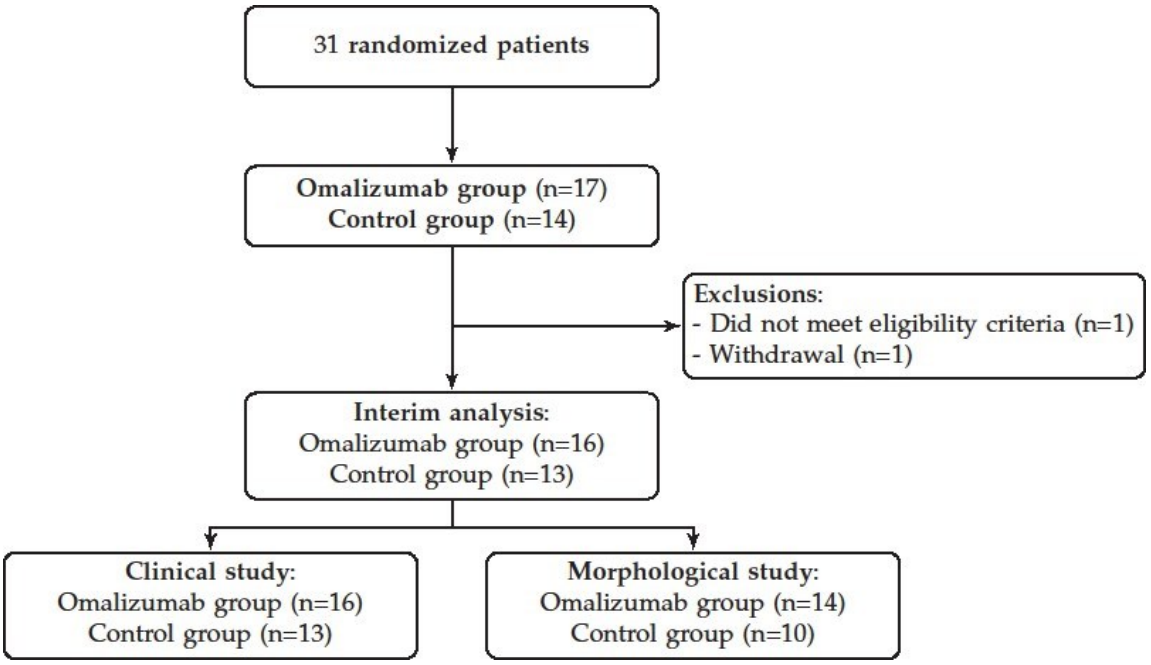

31 randomized patients
Omalizumab group (n=17)
Control group (n=14)
Exclusions:
- Did not meet eligibility criteria (n=1)
- Withdrawal (n=1)
Interim analysis:
Omalizumab group (n=16)
Control group (n=13)
Clinical study:
Omalizumab group (n=16)
Control group (n=13)
Morphological study:
Omalizumab group (n=14)
Control group (n=10)

Figure 2.

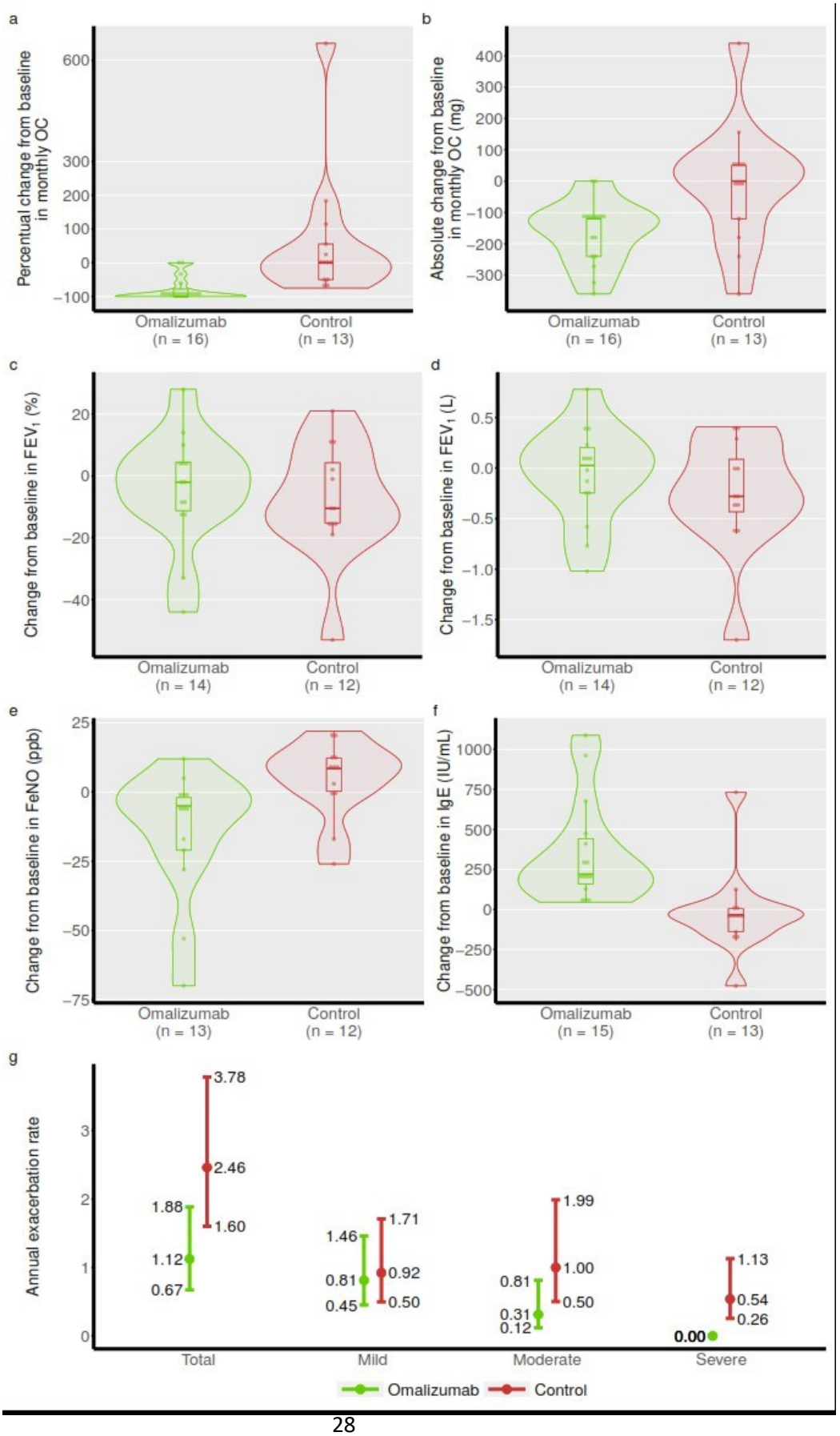

a
Percentual change from baseline in monthly OC
600
300
200
100
0
−100
Omalizumab (n = 16)
Control (n = 13)
b
Absolute change from baseline in monthly OC (mg)
400
300
200
100
0
−100
−200
−300
Omalizumab (n = 16)
Control (n = 13)
c
Change from baseline in FEV1 (%)
20
0
−20
−40
Omalizumab (n = 14)
Control (n = 12)
d
Change from baseline in FEV1 (L)
0.5
0.0
−0.5
−1.0
−1.5
Omalizumab (n = 14)
Control (n = 12)
e
Change from baseline in FeNO (ppb)
25
0
−25
−50
−75
Omalizumab (n = 13)
Control (n = 12)
f
Change from baseline in IgE (IU/mL)
1000
750
500
250
0
−250
−500
Omalizumab (n = 15)
Control (n = 13)
g
Annual exacerbation rate
3
2
1
0
3.78
2.46
1.88
1.60
1.12
0.67
1.71
1.46
0.92
0.81
0.50
0.45
1.99
1.00
0.81
0.50
0.31
0.12
1.13
0.54
0.26
0.00
Total
Mild
Moderate
Severe
Omalizumab
Control

Figure 3.

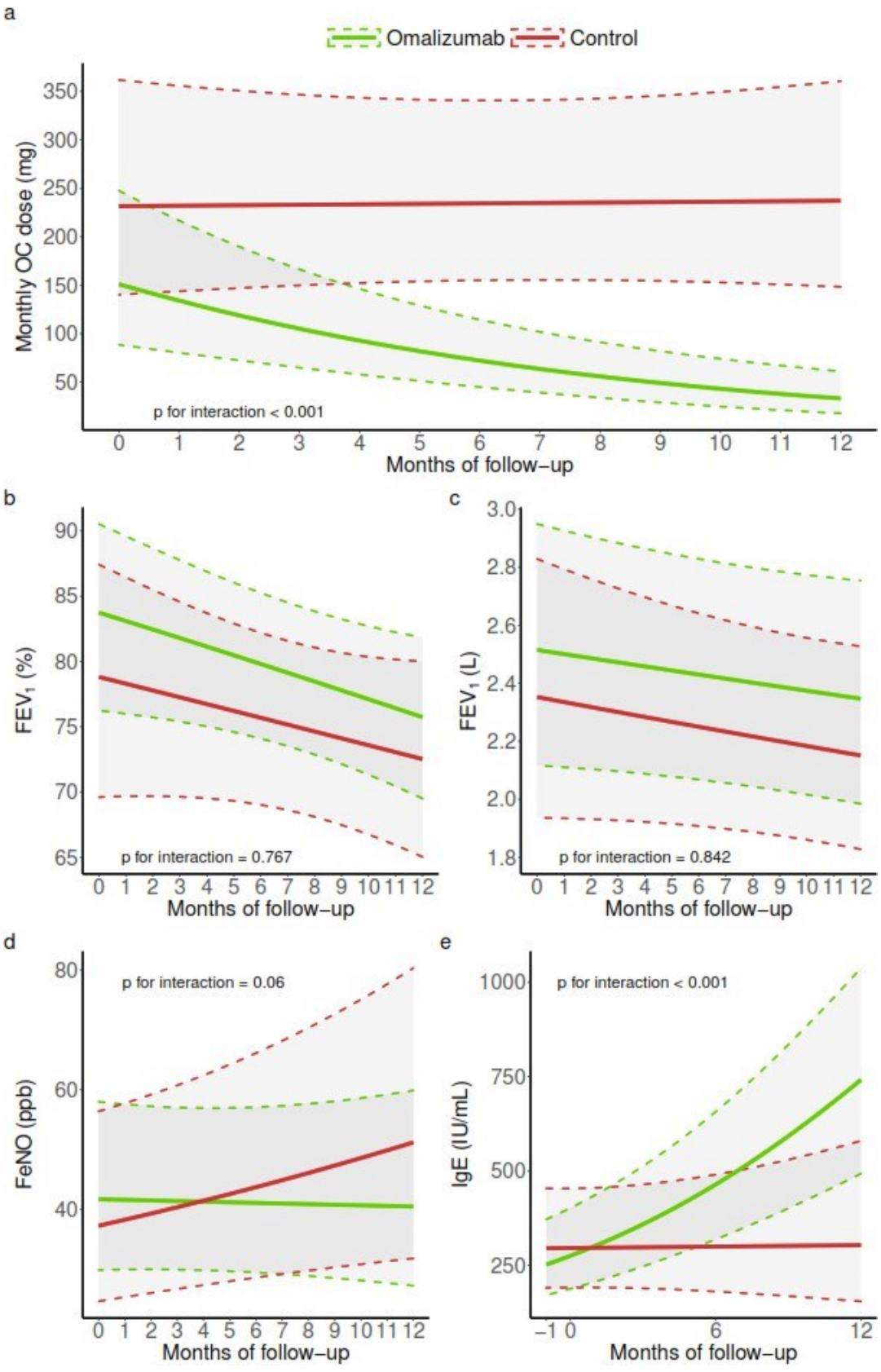

a
Omalizumab
Control
Monthly OC dose (mg)
350
300
250
200
150
100
50
p for interaction < 0.001
0 1 2 3 4 5 6 7 8 9 10 11 12
Months of follow−up
b
FEV1 (%)
90
85
80
75
70
65
p for interaction = 0.767
0 1 2 3 4 5 6 7 8 9 10 11 12
Months of follow−up
c
FEV1 (L)
3.0
2.8
2.6
2.4
2.2
2.0
1.8
p for interaction = 0.842
0 1 2 3 4 5 6 7 8 9 10 11 12
Months of follow−up
d
FeNO (ppb)
80
60
40
p for interaction = 0.06
0 1 2 3 4 5 6 7 8 9 10 11 12
Months of follow−up
e
IgE (IU/mL)
1000
750
500
250
p for interaction < 0.001
−1 0 6 12
Months of follow−up

Figure 4.

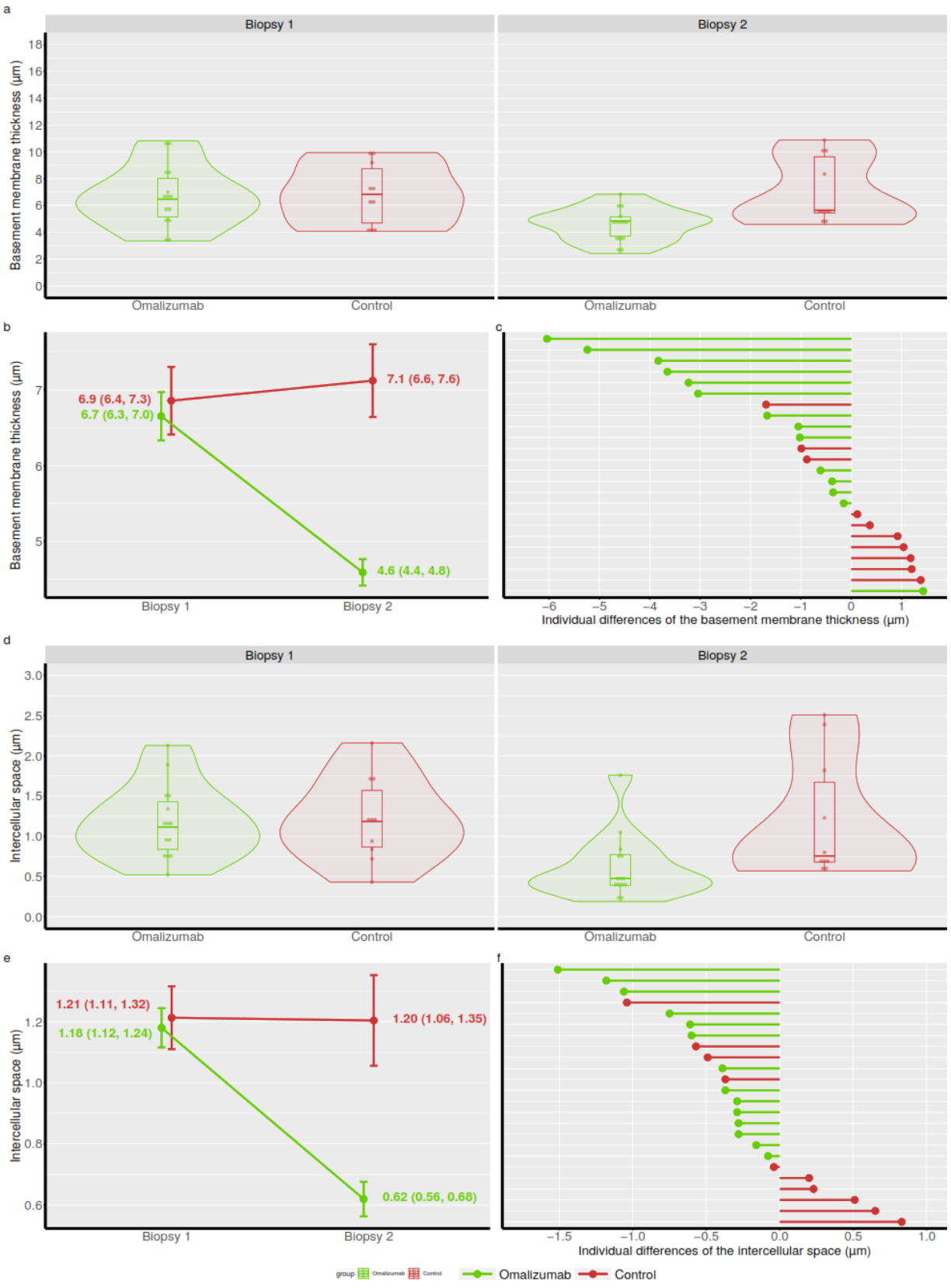
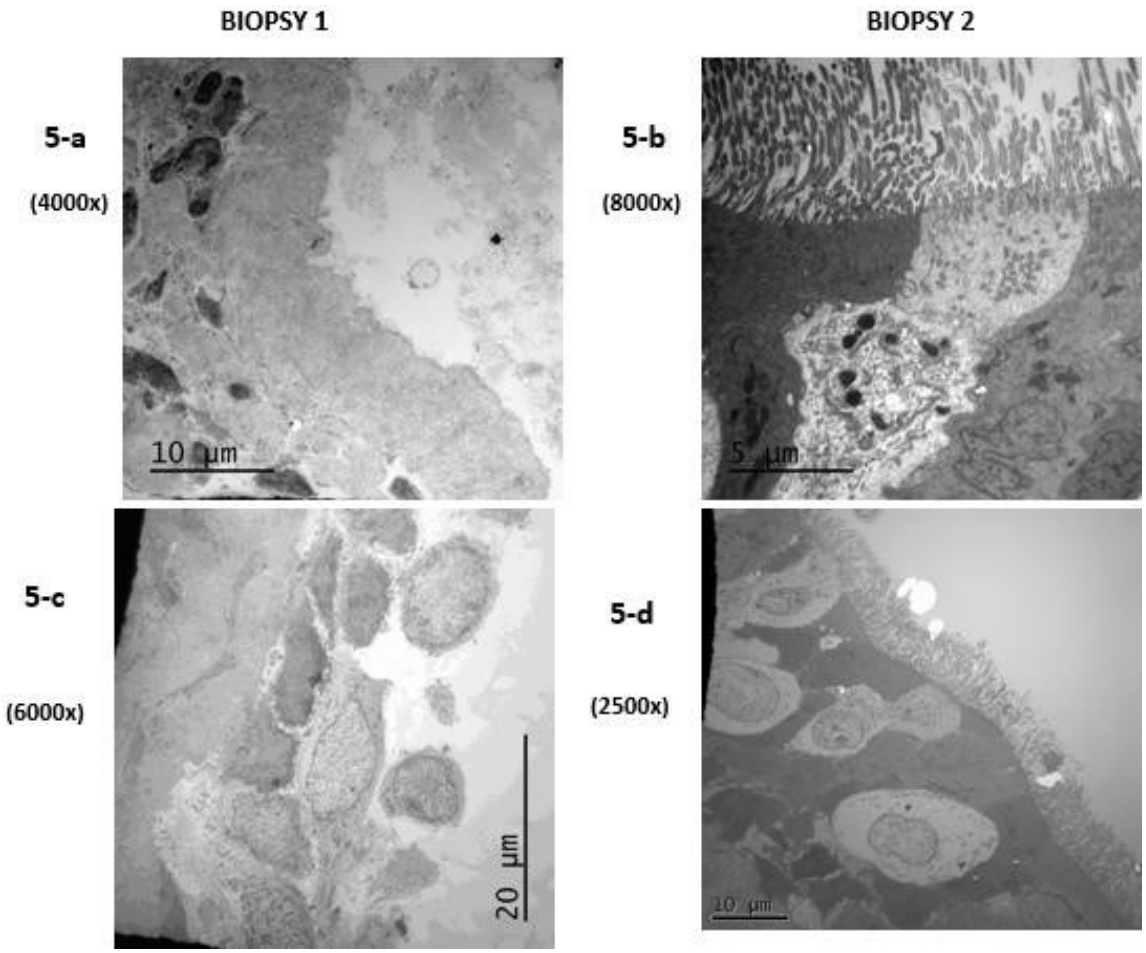

a
Biopsy 1
Biopsy 2
Basement membrane thickness (μm)
Omalizumab
Control
b
Basement membrane thickness (μm)
6.9 (6.4, 7.3)
6.7 (6.3, 7.0)
7.1 (6.6, 7.6)
4.6 (4.4, 4.8)
Biopsy 1
Biopsy 2
c
Individual differences of the basement membrane thickness (μm)
d
Biopsy 1
Biopsy 2
Intercellular space (μm)
Omalizumab
Control
e
Intercellular space (μm)
1.21 (1.11, 1.32)
1.18 (1.12, 1.24)
1.20 (1.06, 1.35)
0.62 (0.56, 0.68)
Biopsy 1
Biopsy 2
f
Individual differences of the intercellular space (μm)
group
Omalizumab
Control

Figure 5.


BIOPSY 1
BIOPSY 2
5-a
(4000x)
10 µm
5-b
(8000x)
5 µm
5-c
(6000x)
20 µm
5-d
(2500x)
10 µm

Declarations:

-Funding.

Grants from SEPAR 845-2009/ FUCAP 2008/FUCAP 2009

-Conflicts of Interest.

CD declares having received financial aid for travel support and speakers bureaus from Novartis, Sanofi, GSK, TEVA, MSD, Esteve, Almirall, Astra-Zeneca, Chiesi, Menarini, Pfizer, Ferrer, Stallergenes, ALK-Abelló, Allergy therapeutics, Hall Allergy, Inmunotek, Roxall.

RMM, CF, and FG declare that they have no conflicts of interest.

No authors have any conflict of interest in relation to this study.

-Availability of data and material.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

-Ethics approval –

The study was approved by the Institutional Review Boards of both hospitals. Written informed consent was obtained from all patients. The study was registered with EudraCT number 2009-010914-31. The study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

-Consent to participate.

"Written informed consent was obtained from all patients"

-Consent for publication Written informed consent was obtained from all patients"

-Code availability. NA

-Authors' contributions:

CD: Conceptualization, funding acquisition, data curation, methodology, project administration, resources, supervision, verification, visualization, writing original draft.

RMM: Conceptualization, data curation, methodology, resources, supervision, verification, visualization, writing original draft.

FJGB: data curation, resources, verification, writing -review & editing.

CF: Formal Analysis, Methodology, Visualization, Writing -review & editing.

FP: data curation, resources, verification, Writing -review & editing.

All authors have read and approve the final version of the manuscript, and agree to be accountable for the work.

-FINANCIAL DISCLOSURE

CD declares having received financial disclosure for travel support and speakers bureaus from Novartis, Sanofi, GSK, TEVA, MSD, Esteve, Almirall, Astra-Zeneca, Chiesi, Menarini, Pfizer, Ferrer, Stallergenes, ALK-Abelló, Allergy therapeutics, Hall Allergy, Inmunotek, Roxall.

FJGB declares having received consulting fees and speakers bureaus from ALK, Astra-Zeneca, Bial, Chiesi, Gebro Pharma, GlaxoSmithKline, Menarini, Novartis, Rovi, Roxall, Sanofi, Stallergenes-Greer, Teva and participated in advisory boards for ALK, Astra-Zeneca, GlaxoSmithKline, Menarini, Novartis, Sanofi, Teva.

RMM, CF, and FG declare that they have no conflicts of interest.

No author declares a conflict of interest in relation to this study.

OMALIZUMAB IN SEVERE ASTHMA: EFFECT ON ORAL CORTICOSTEROID EXPOSURE AND REMODELING.

A randomized open-label parallel study.

Short title: Omalizumab in severe asthma: effect on oral corticosteroid exposure and remodelling

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Acknowledgements.

We thank Anna Garrit for her technical assistance in electron microscopy laboratory (Department of Morphological Sciences- UAB), Natàlia Martínez, nurse at the Respiratory Service (Corporació Parc Taulí-UAB) and Dr. Vicente Marco-Molina (Servei d'Anatomía Patològica, Hospital Quirón (Barcelona).

Key points:

- The study shows the OC-sparing effect and restorative capacity on bronchial epithelium of a biological treatment.
- Contrary to previously held beliefs, it shows that bronchial remodeling in patients with severe asthma is reversible with biologic therapy despite a reduction in the dose of oral corticosteroids.
- It allows reinterpretation of the histological changes found, so that the thickening of the basement membrane appears to be a protective reaction of the epithelium to exert the barrier function lost with the damage of the disease.
- It reinforces the current idea of the importance of the bronchial epithelium in the pathophysiology of asthma (asthma as "epithelial driven disease").

Abstract word count: 341

Body text word count: 4573

Abstract

Introduction: Data on the clinical efficacy and remodeling of omalizumab therapy in patients on oral corticosteroids (OC) are limited.

Objective: The purpose of the study is to show that in patients with corticosteroid dependent asthma, omalizumab is a corticosteroid-sparing therapy able to inhibit airways remodeling and to reduce disease burden (lung function impairment, exacerbations).

Methods: CHOC is a randomised open-label study evaluating the addition of omalizumab to the standard of care in patients with severe asthma receiving oral corticosteroids. The primary endpoint was represented by the change in OC monthly dose by the end of treatment and secondary endpoints included spirometry changes, airway inflammation (FeNO), number of exacerbations and airways remodelling assessed by bronchial biopsies studied by transmission electron microscopy. As safety variable, adverse effects were collected.

Results: Efficacy was assessed for 16 patients in the omalizumab group and 13 in the control group. The final cumulative mean monthly OC doses were 34.7 and 217 mg for the omalizumab and control group respectively; the mean difference between groups adjusted for baseline was -148.1 [95% CI: -243.6, -52.5] (p=0.004). OC withdrawal: 75% vs. 7.7%; p=0.001).
Omalizumab provided: a slowing of FEV1 loss (70 mL vs 260 mL), a significant decrease in FENO values and a reduction in the annual relative risk of clinically significant exacerbations of 54%. The treatment was well tolerated. The morphological study showed a significant decrease in basement membrane thickness in the omalizumab group (6.7 vs. 4.6 µm) compared to controls (6.9 vs. 7. µm) (mean difference between groups adjusted for baseline = -2.4 [95% CI: -3.7, -1.2] p<0.001), as well as a decrease in intercellular spaces (1.18 vs. 0.62 µm and 1.21 vs. 1.20 µm]; p=0.011) respectively). A qualitative improvement was also observed in the treated group.

Conclusions: Omalizumab showed a marked OC-sparing capacity and was associated with an improvement in clinical management that correlated with bronchial epithelial repair. In OC-dependent asthma, reversibility of remodeling is possible; the concepts that basement membrane enlargement is detrimental and that chronic airway obstruction is systematically irreversible are outdated (EudraCT: 2009-010914-31).

1.- INTRODUCTION.

Over the last 15 years, a new drug family has emerged for the treatment of severe asthma: the monoclonal antibodies (mAb). Initially recommended as the last step for treatment of severe uncontrolled asthma, recent guidelines now place mAb administration in front of that of oral corticosteroids.

The first mAb to be marketed was omalizumab, a humanized murine anti-IgE for allergic asthma (1,2). The pivotal studies were published at the beginning of this century (3-6). Since then, several other mAbs have been marketed (7). The requirements for registration have evolved and become more strict: regulatory agencies now expect companies to demonstrate clinical benefits (decrease in exacerbations and hospital admissions, improvement of pulmonary function testing, inhaled corticosteroid sparing effect) not only in moderate/severe asthma but in oral corticosteroid (OC) dependent patients as well (8-10).

To date, a vast amount of papers have been published on omalizumab and have shown it to be well tolerated and clinically useful in the management of moderate/severe allergic asthma (3-6). In spite of this, the clinical information regarding certain aspects of the drug's activity is limited and the information on its performance in some specific situations which have in fact been assessed in studies describing the development of more recent mAbs is absent. Moreover, the information about the potential effects of mAbs on remodeling is scarce or absent.

To fill this gap, we designed a study to evaluate the **C**linical and **H**istological impact of treatment with **O**malizumab in severe allergic oral **C**orticosteroid dependent allergic asthma patients (the CHOC study).

2.- MATERIAL AND METHODS.

The hypothesis to be tested was the following: Omalizumab, an anti-IgE recombinant humanized monoclonal antibody, is a well-tolerated drug that aids the management of allergic corticosteroid-dependent asthma patients. Its strong points are: a) It allows a reduction in OC intake; b) It improves clinical management of patients by decreasing exacerbations and stabilizing pulmonary function; c) It reverses histopathological changes in the bronchial wall, and also the chronic inflammation.

2.1 Population*:* Patients were recruited from the asthma out-patient clinics of the Pulmonary Services at two hospitals (Hospital Parc Taulí and Hospital Clínico Universitario de Santiago de Compostela, Spain). Briefly, the study included adult allergic corticodependent asthma patients. The inclusion/exclusion criteria are described in the online resource 1. Briefly, the population studied comprised adult allergic OC-dependent asthma patients eligible for omalizumab treatment who were not receiving specific immunotherapy.

2.2 Outcomes*: Primary efficacy outcome*: a) Change in OCS monthly dose by the end of treatment; *Secondary efficacy outcomes*: a) Spirometry changes; b) Airway inflammation (fraction exhaled of nitric oxide [FeNO]; c) Number of exacerbations; d) Reversibility of the histological changes in the bronchial mucosa. *Safety outcomes*: a) Adverse events related to drug treatment.

2.3 Type of study*:* Phase IV, open-label randomized parallel study (EudraCT: 2009-010914-31) with two groups treated with best standard care receiving OC: the omalizumab group receiving omalizumab as add-on therapy and the control group not receiving omalizumab.

Although the study had an open design, the pathologist and the clinicians were blinded to the clinical and pathological findings respectively.

***2.4 Stages of the study**:* The study comprises three stages (online resource 2).

2.4.1 Stabilization period: The stabilization period was planned to last at least three months. During this period patients received the best standard care for asthma according to GINA (Salmeterol /Fluticasone 50/500 Acuhaler® bid, montelukast 10 mg/once a day, OC and salbutamol on request). At the beginning of this period, patients receiving prednisolone were switched to 6-methyl-prednisolone (6-MP); 4 mg of 6-MP were considered equivalent to 5 mg of prednisolone. OC doses were progressively tapered (2 mg/day of 6-MP every two weeks) until a decrease of 10% or more in the forced expiratory volume in one second (FEV_1) was observed. The daily dose of 6-MP was then increased by 2 mg every two weeks until the FEV1 returned to its previous levels and the process was repeated. At the third failed attempt to reduce the OC dose, we considered that the minimum dose required had been attained. A written informed consent was obtained.

2.4.2 Run-in period: A 4-week period, before randomization after the patient had agreed to participate in the trial.

2.4.3 Follow-up/Treatment period: Patients were randomized to receive or not receive a subcutaneous dose of omalizumab. Omalizumab dose was based on body weight and initial serum immunoglobulin E (IgE) (approximately equal to 0.016mg/Kg x body weight (in kg) x IgE (in International Units/mL) per 4 weeks for a 12-month period. During the treatment period, the oral corticosteroid decrease protocol followed was the same than during the stabilization period (6-MP was progressively tapered (2 mg/day every two weeks) until FEV_1 fell by 10% or more; the 6-MP dose was then increased to the previous dose until the FEV1 returned to its previous levels). The procedure was repeated throughout the follow-up.

2.5 Randomization: Patients were assigned to their group according to a randomization list linked to a treatment arm. Patients were allocated to one or other arm according to a ratio 1:1.

2.6 Study protocol*:*

2.6.1 Definition of exacerbations: Exacerbations were classified into three groups. Mild exacerbations were those that required occasional (< 3 days) increase of OC and/or antibiotics. Moderate exacerbations included increase of OC intake (≥ twice the baseline dose) during ≥ 3 days and/or antibiotics + unscheduled ambulatory visit. Severe exacerbations included increased OC intake (≥ twice the baseline dose) ≥ 3 days and/or antibiotics + hospital admission.

2.6.2 Measurements:

At the beginning and end of the study, the following tests were performed in each patient: skin prick test, spirometry, FeNO measurement, blood analysis (including IgE), bronchoscopy with bronchial biopsies for morphological study (transmission electronic microscopy [TEM]).

After six months: blood analysis including IgE.

Monthly: spirometry, FeNO measurement, daily OC dose, monthly accumulated OC dose, recording of exacerbations and side-effects.

Technical details of every test can be found in the online resource 3 and 4.

Morphological study of bronchial biopsies (Transmission electron microscopy-TEM).

The processing of biopsies for electron microscopy is described in the online resources 5-8. We studied the degree of the epithelial damage focusing on the thickness of the basement membrane, the intercellular space of the epithelium and characteristics of the epithelium and cilia following standardized methods (11, 12). The measurements were performed twice by the same EM expert, in two different time periods separated by a minimum of three months.

2.7 Sample size calculation. The sample size for this study was 28 randomized patients in each of two treatment groups. The sample size was calculated to achieve a power greater than 80% using a two-sided z-test at a significance level of 5% and based on the primary efficacy endpoint –percentual change from baseline in monthly oral corticosteroids at the end of follow-up–, expecting to find decreases of 50% (standard deviation [SD]=50) in the omalizumab group and 10% (SD=50) in the control group.

An interim efficacy analysis was planned after 50% of recruitment, that was oversaw by an independent data monitoring committee. Test boundaries were determined using the Pocock spending function (see Table 1s in online resource 9).

2.8 Statistical analysis.

Between-group differences in changes (end minus baseline) of continuous outcomes (both clinical and morphological) were analysed by means of linear regression models adjusting for basal measures. End-study measures from the morphological study were analysed by means of logistic regression models for dichotomous outcomes and proportional odds ordinal logistic regression models for ordered outcomes, adjusting for basal measures. Longitudinal analysis of all clinical measurements collected throughout the months of follow-up was performed by means of generalised linear mixed effects models, including interaction term between study group and follow-up time in all models allowing the groups' changes to differ over time. Annual exacerbation rate ratios (total and by severity) were estimated using negative binomial generalised linear models.

R version 4.2.0 was used for all analyses (13). The mixed model function of the **GLMMadaptive** package (14) was used to fit the mixed-effects models; the vglm function of the **VGAM** package (15) was used to fit ordinal logistic models; the glm.nb function of the

MASS package (16) was used to fit negative binomial models; and results were visualized using the **ggplot2** package (17).

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

2.9 Institutional review board approval.

The study was approved by the Institutional Review Board of both hospitals. Written informed consent was obtained from every patient. The study was registered with EudraCT number 2009-010914-31. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

3.- RESULTS.

Initially, 31 patients were randomized, of which two were excluded. The study was stopped for efficacy, with 16 patients in the omalizumab group and 13 patients in the control group, and proceeded to the analysis of the rest of the data (figure 1).

3.1 **Clinical results**. At entry, both groups exhibited similar demographic and clinical characteristics (Table 1).

3.1.1 Oral corticosteroid use. At the end of follow-up, mean monthly accumulated OC doses were 34.7 mg (SD=81.6) and 217 mg (SD=186) for the omalizumab and the control group, respectively. These figures corresponded to mean percentual changes from baseline of 79% less (SD=36) in the omalizumab group and 61% more (SD=191) in the control group (figure 2a) and the mean difference between groups adjusted for basal was -148.1 [95% CI: -243.6, -52.5] (p=0.004). Mean absolute differences were -163.8 mg (SD=102) and -8.8 mg (SD=196) for the omalizumab and the control group, respectively (figure 2b); when adjusted for basal measures,

mean difference between groups was -170.5 [95% CI: -264.5, -76.5] (p<0.001). After removing extreme values from the control group as sensitivity analysis, the adjusted mean differences were -96.1 [-139.4, -52.9] (p<0.001) in percentual changes and -132.7 [-195.9, -69.5] (p<0.001) in absolute changes. Twelve (75%) patients in the omalizumab group and only 1 (7.7%) patient in the control group withdrawn OC treatment (p = 0.001).

3.1.2 Pulmonary function tests. Regarding pulmonary function, at the end of follow-up the control group lost 260 mL (SD=580) while the omalizumab group only lost 70 mL (SD=480) from baseline (mean difference between groups adjusted for basal = 226.2 [95% CI: -185.7, 638.1], p=0.268). In percent scale, FEV_1 decreased 7.9% (SD=19.1) and 4.3% (18.3) in the control and the omalizumab group, respectively (mean difference between groups adjusted for basal = 2.79 [95% CI: -10.3, 15.9], p=0.662) (figures 2c-d).

3.1.3 Airway inflammation. Significant difference between groups was found in change from baseline in airway inflammation measured by FeNO values (mean difference between groups adjusted for basal = -17.3 [95% CI: -25.6, -8.94], p<0.001), with a reduction of 14.8 ppb (SD=23.5) in the omalizumab group while the control group experienced an increment of 4.3 ppb (SD=14) (figure 2e). Note that mean final FeNO value in the omalizumab group was 17.7 parts per billion (ppb) (normal value).

3.1.4 Immunoglobulin E changes. Significant difference between groups was also found in change from baseline in IgE values (mean difference between groups adjusted for basal = 425.8 [95% CI: 199.5, 652.0], p<0.001), observing a mean decrement of 20.8 IU/mL (SD=268) in the control group and a mean increment of 355 IU/mL (SD=320) in the omalizumab group (Figure 2F), equivalent to a mean ratio of 3.8 compared to baseline. Additional data are given in the online resource 10 (figures 1s, 2s and 3s).

3.1.5 Exacerbations. The estimated annual rates of clinically significant exacerbations were 1.12 (95% CI = 0.67, 1.88) in the omalizumab group and 2.46 (95% CI = 1.6, 3.78) in the control group figure 2g). Thus, compared with the control group, the annual exacerbation rate ratio was 0.46 (95% CI = 0.23, 0.89). The annual relative risk reduction was 54%. By severity, the annual rate ratio for mild exacerbations was 0.88 (95% CI = 0.37, 2.1); and for moderate exacerbations was 0.31 (95% CI = 0.09, 0.97). No severe exacerbations were observed in the omalizumab group during follow-up. Mean duration of the exacerbations was 13 days (SD=14.8) in the omalizumab group and 31 days (SD=33.6) in the control group (p=0.093). Additional data are given in the online resource 10 (figures 4s and 5s).

3.1.6 Longitudinal trends of the clinical outcomes by study groups. The longitudinal analysis showed that lung function, airways inflammation and OC cumulative monthly dose showed constantly more favourable trends in omalizumab group (figure 3) (interaction term p<0.001). As expected, IgE levels increased over time for the omalizumab group but no for the control group (see also figure 6s in the online resource 10).

3.2 Morphological study.

No statistically significant differences were observed between the two groups at entry in any of the parameters studied.

3.2.1 Thickness of the basement membrane (BM). Figure 5 shows the thickening of BM. The omalizumab group showed a marked decrease in BM thickness, while controls presented a slight increase (mean difference between groups adjusted for basal = -2.4 [95% CI: -3.7, -1.2], p<0.001). The decrease in BM thickness occurred in 13 out of 14 patients (92.9%) of the omalizumab group and in only 3 out of 10 (30%) of the control group (figure 4).

3.2.2 Intercellular space. Similar to thickness of the BM, the omalizumab group showed a marked decrease in intercellular spaces, and controls remained stable (mean difference between

groups adjusted for basal = -0.56 [95% CI: -0.98, -0.14], p=0.011). In the omalizumab group all patients presented reductions in the intercellular space compared to only 5 out of 10 controls (figures 4 and 5).

3.2.3 Damage to the epithelium and cilia description. We observed considerable detachment of the bronchial epithelium(fig ure 5). In the first biopsy (at entry), patients in the omalizumab group showed more severe damage in the epithelium (two patients showed a total loss of the columnar epithelium) than patients in the control group, although there were no statistically significant differences between groups (online resource 10, figures 7s). Statistically significant differences were found between groups at the end of the study adjusting for basal damage (p=0.0499). Additionally, a higher percentage of improvers during the follow-up in the omalizumab group (64.3%) than the control group (20.0%) was observed (online resource 10, figures 8s). The two patients of the omalizumab group who showed at entry a total loss of the columnar epithelium showed reappearance of the columnar cells although some detachment was still present.

Regarding the extent (focal or extensive) of the damage to the epithelium, the percentage of patients in the omalizumab group with focal involvement increased from 35.7% to 50% at the end of the study, while in the control group the percentage of patients showing extensive involvement notably increased from 70% to 90% (online resource 10, figures 9s). Nevertheless, adjusting for basal measures, no statistically significant difference between groups at the end of the study was observed (p=0.064). Finally, regarding the qualitative evaluation of the changes observed in the cilia, the percentage of patients in the omalizumab group with no absence or focal absence increased from 35.7% to 71.4% at the end of the study, while in the control group these figures decreased from 50% to 40%. Adjusting for basal measures, a

statistically significant difference between groups at the end of the study was observed (p=0.038).

In the initial and final biopsies, we observed some ultrastructural abnormalities such as axoneme bubble, presence of only one singlet and supernumerary singlets, presence of compound cilia, and ciliary oedema and ciliary disorientation. Abnormalities present in the first biopsy persisted in the second biopsy, independently of the group. When ciliogenesis occurred in the second biopsy (mainly in the omalizumab group), ultrastructural abnormalities were rare, except for the presence of some ciliary disorientation (Figure 10s).

3.3 Side effects. Regarding side effects, the treatment was well tolerated and there some side effects reported in the online resource 11. Only arthromyalgia, that persisted at the end of the treatment could be considered a drug-related side effect. None patient abandoned the treatment.

4.- DISCUSSION

The purpose of our study was to elucidate the clinical and histological benefits of a one-year treatment with omalizumab in the most severe population of asthma patients, namely those who are OC-dependent. Omalizumab has demonstrated its ICS-corticosteroid sparing capacity, but there were no randomized clinical trials designed to show its OCS-sparing capacity. The Cochrane review (18) concluded that omalizumab treatment was associated with a significant probability of reducing inhaled corticosteroid doses or of withdrawing them completely. The mean dose reduction was -118 μg equivalent of beclomethasone dipropionate. In the subgroup of patients who received oral steroids, it was not clear whether this benefit occurred. In a subgroup analysis of an open-label parallel group study, Siergiejko et al (19) found that at week 32, 62.7% of patients in the treated group were able to stop or reduce OCs compared to 30.4% of controls. The real-life study eXpeRience (20) found a relative reduction of 50% at month 24, and APEX (a UK retrospective study) found a 34% reduction in the mean total quantity of OCs prescribed per year and complete cessation of OC use after 12 months in 48% of patients (21). In a two-year observational prospective study (22), we found that omalizumab allowed OC withdrawal in 74.2% of patients. It is normally considered that real-life studies obtain better results than randomized clinical trials, but CHOC presented results of the same order as the best real-life studies.

One of the key parameters used by regulatory agencies for registering a mAb is its capacity to decrease the rate and the severity of exacerbations. The INNOVATE study (23) showed that the clinically significant exacerbation rate (the primary efficacy variable), adjusted for an observed imbalance in exacerbation history, was 0.68 with omalizumab and 0.91 with placebo (26% reduction) during the 28-week treatment phase. Without adjustment, a similar magnitude of effect was seen (a reduction of 19%), although it did not reach statistical significance. In our

study, the annual exacerbation rate ratio showed a 54% reduction of total exacerbations. Regarding severe exacerbations, the INNOVATE (23) study showed a 50% annualized reduction. In our study none of the omalizumab patients presented severe exacerbations during follow-up. In addition, in our study the duration of the exacerbation was halved. To summarize, our data show that omalizumab helped to reduce exacerbations, especially the most severe ones. Two real-life studies involving OC-dependent patients (eXpeRience registry (20) with 28.1% of patients receiving OC and in the Xpertise trial (24) with 46%) showed a decrease in exacerbations of above 80%

In addition to exacerbations, some studies have measured the emergency room visits or hospitalizations. The INNOVATE (23) study, in which OC-dependent patients accounted for 22% of the sample and 78% were on inhaled corticosteroid, reported an annualized 44% reduction in emergency room visits. In a real-life study, Molimard et al (25) found a 65% reduction in annual emergency visits following omalizumab therapy. In Germany, in a population of 280 patients of whom 46% were OC-dependent, Korn et al (24) reported an 82% reduction in exacerbations and a 78% reduction in hospitalizations. In our study, emergency visits were addressed indirectly. Exacerbations were defined as severe if they required hospital admission. The absence of severe exacerbations in the omalizumab group indirectly reflects the absence of hospitalizations despite the decrease or withdrawal of OCs.

Another purpose of a biological treatment is to improve or minimize the loss of lung function. In OC-dependent patients, the main point is to demonstrate this at the same time as the reduction in OC use. The Normansell Cochrane review (18) included two studies with 732 participants and showed a significant difference in the mean improvement of FEV_1 compared to baseline in moderate/severe asthma (67.29 mL). The eXpeRience trial (20) showed an improvement in FEV_1 of 8.7% after two years. No information is available on pulmonary function testing

changes in OC-dependent patients. We observed FEV_1 losses of 70 mL in the treated group and 260 mL in the control group. We regard these differences as being clinically relevant. After one year of treatment, omalizumab was able to slow down the loss of lung function in the most severe group of patients although the loss continued to be higher than expected in a healthy population. There are two possible explanations for this finding: the limited follow-up (maybe a longer treatment period might bring down the loss of FEV1 to within normal limits) or the limited effect on the thickness of the smooth muscular layer.

The changes in airway inflammation are represented by FeNO values. FENO reflects the activity of IL13 which is released by Th2 lymphocytes as well as innate lymphoid cells. Significant changes were observed at the end of the follow-up. Moreover, in the omalizumab group, the final mean FeNO value was within normal limits (below 25 ppb, the value specified by GINA for diagnosis of a T2 asthma). Thus, omalizumab treatment clearly contributed to downregulating the bronchial inflammation, despite the decrease in OC intake. In the control group, the increased FENO values reflect the activity of the Th2 cascade and/or the persistence of the stimulus of alarmins whose release does not diminish because the integrity of the bronchial ciliated epithelium does not improve.

Finally, as expected, there was a marked increase in blood IgE concentration, reflecting the treatment with the anti-IgE blocker (22). Regarding safety, no relevant side effects related to treatment occurred that limited the treatment with omalizumab.

The histological study of the bronchial biopsies was performed by electronic microscopy. (26, 27). In the last two decades, the histopathological changes that occur in asthma have attracted attention. Initially, inflammation was identified as the main pathophysiological cause of chronicity of the disease. Later, attention has focused on the disruption of bronchial epithelium

(26), and the loss of cilia and thickening of the basement membrane of the epithelium (27) (especially the reticular layer).

The histological improvement observed in our study was dramatic. At entry, a marked denudation of the bronchial mucosa was observed, with loss of cilia, increased intercellular space, and cell loss. The BM was also markedly thickened, but treatment with omalizumab reduced its thickness. BM thickening has traditionally been considered to be irreversible once it occurs. Riccio et al (28) in a study without a control group already suggested that some degree of reversibility could occur as a result of omalizumab treatment. Our study is the first to stablish that this benefit occurs after omalizumab in comparison with a control group. The BM enlargement probably develops as a protective mechanism, with the allergen or other impurities on one side and the antigen presenting cells on the other. The thicker the BM, the less likely the interaction between external noxae and subepithelial cells. Considering that the barrier function of the bronchial epithelium is markedly impaired, the thickening of the BM may contribute to regulating the deterioration of asthma. The bronchial cells are normally next to each other and there is no intercellular space, and desmosomes help to maintain the structure and the barrier function of the epithelium. One of the first disorders that occurs in cells is the loss of cilia and subsequently, the loss of their function as cleaners of the mucosa, meaning that pollutants, allergens, and virus are able to remain in the airway. The appearance of intercellular space is a sign that precedes the absolute denudation of the mucosa. In some patients the cell loss is total; the BM comes into direct contact with the airway lumen and is exposed to pollutants, virus and allergens. At this point, the functions of the bronchial epithelium break down. Although chronic treatment with OC has shown a certain reparative effect in some patients, the benefits observed in most of the patients receiving omalizumab were notably greater. These morphological changes should help to improve asthma control, decrease exacerbations, and achieve other

clinical benefits, in some cases allowing the withdrawal of the drug (29). Thus, our results challenge the belief that chronic remodeling is irreversible, that the thickening of the BM is harmful, and that chronic airway obstruction, when present, is irreversible (30).

There are several possible explanations for these morphological changes. “In vitro” studies have shown that several cells, especially smooth muscle cells, have receptors for IgE. This causes hyperplasia and hypertrophy of the smooth muscle layer (31). The presence of IgE has also been shown to favour the depositing of collagen fibers and extracellular matrix (32). The blockade of the binding of IgE to its receptor prevents or postpones these deposits (32). The remodeling caused by the presence of IgE can also be prevented by omalizumab (33). This has a synergistic effect with the blockade of acetylcholine which also favours the deposition of extracellular matrix (34). It should be noted that the influence of IgE in the remodeling process occurs in the absence of allergens. The blockade of IgE postpones these changes and the histological changes are manifested in a decrease in the thickness of the bronchial wall (35) or, in some cases, a complete reversibility of the airway obstruction (36). Finally, the repair of the bronchial epithelium contributes to a decrease in the release of alarmins, thus limiting their harmful effect.

The most important limitation of the study is the lack of placebo in the control group. We could not obtain an oily solution similar to omalizumab and thus we would have had to use a placebo that would have had a very different appearance. Since the nurses collaborating in the study in charge of treatment administration would have easily differentiated the placebo from omalizumab, and the treatment was administered at the hospital day unit (where patients on biologics are usually treated) the possibility that patients would have identified the treatment/placebo was very high. Therefore, we thought it was better to plan the study as a parallel randomized but open-label study. To minimize the influence of the lack of placebo in

the interpretation of the results, the pathologist, as well as the clinicians, were blinded to the clinical and pathological findings, respectively.

5.- Conclusions. In summary, CHOC fills in some of the gaps in the literature regarding the benefits omalizumab can offer in OC-dependent allergic asthma patients. It is the first randomized study to show the clinical benefits of omalizumab including the slowing down the loss of respiratory function despite the OC decrease. Moreover, it is also the first study to show the histological abnormalities that occur in severe allergic asthma patients with chronic OC treatment, and the capacity of omalizumab to facilitate the repair of the bronchial epithelium, given that some of the patients returned to normality. It thus prompts us to rethink the interpretation of remodeling changes, and to abandon the widespread idea that chronic remodeling is irreversible. The histological recovery of the bronchial mucosa can help to explain the improvement in clinical management of the patients (35) and also the feasibility of withdrawing omalizumab in certain patients (29). This would be in line with the current view on the importance of the bronchial epithelium in the pathophysiology of severe asthma (37).
The results of CHOC study invalidate the current interpretations of the data. The idea that BM thickening is detrimental can be considered outdated and erroneous. This process occurs in order to replace the barrier or protective function of the bronchial epithelium, which is damaged in asthma patients. Taken together, the results lead us to conclude that omalizumab is a disease-modifying drug. Finally, the concept of remission has recently been introduced. Future research may clarify whether the changes observed in CHOC can help explain the clinical remission achieved by some patients (38).

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TABLES

Table 1. Baseline data for omalizumab group and control group

	Omalizumab group N=16	**Control group N=13**
Age, mean (SD)	47.2 (14.0)	46.5 (13.9)
Women, n (%)	11 (68.8%)	8 (61.5%)
FEV_1 (%), mean (SD)	78.4 (20.2)	79.8 (12.3)
FeNO (ppb), median (P25, P75)	18.0 (14.0, 38.0)	18.5 (14.5, 36.5)
IgE (IU/mL), median (P25, P75)	96.5 (71.2, 169)	145 (80.0, 332)
Monthly OC dose (mg), median (P25, P75)	150 (120, 248)	240 (90.0, 300)
Daily OC dose (mg), median (P25, P75)	4.5 (4.0, 6.5)	6.0 (4.0, 8.0)

FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; IgE, Immunoglobulin E; OC, oral corticosteroid; P25, 25th percentile; P75, 75th percentile; SD, standard deviation.

FIGURE LEGENDS

Figure 1. Study flowchart.

Figure 2. Changes from baseline in clinical outcomes at the end of follow-up and annual exacerbation rate by study group and severity.

FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; IgE, Immunoglobulin E; OC, oral corticosteroid

Figure 3. Longitudinal trends of clinical outcomes by study group

FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; IgE, Immunoglobulin E; OC, oral corticosteroid

Figure 4. Transmission electron microscopy outcomes

Figure 5. Improvement in epithelial damage in the omalizumab group.

Note the changes in the bronchial biopsy before and after one year of omalizumab treatment. Figure 5-a shows the thickening of the BM and the absence of bronchial epithelial cells. Figure 5-b shows the return to normal, disappearance of intercellular spaces and reappearance of cilia.

Figure 5-c shows the initial biopsy from another patient, showing the disappearance of cilia, the appearance of intercellular spaces and denudation of cells. Figure 5-d (12 months after omalizumab treatment) shows a picture of normal ciliated epithelium with palisade cells and reappearance of cilia.

Figure 1.

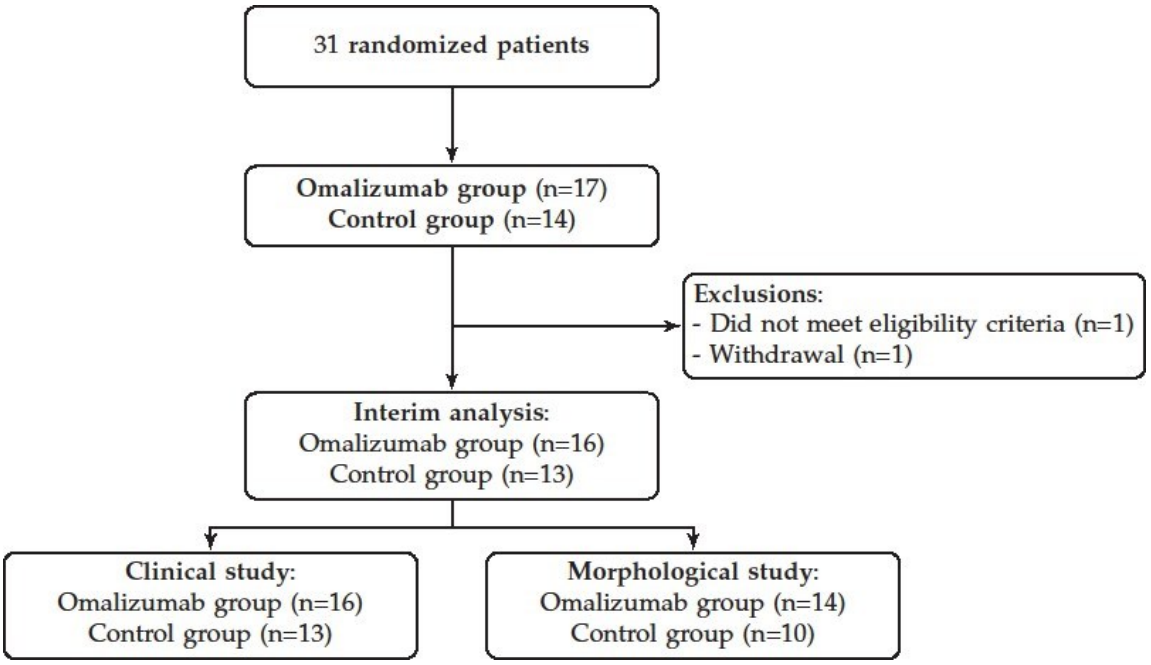

31 randomized patients
Omalizumab group (n=17)
Control group (n=14)
Exclusions:
- Did not meet eligibility criteria (n=1)
- Withdrawal (n=1)
Interim analysis:
Omalizumab group (n=16)
Control group (n=13)
Clinical study:
Omalizumab group (n=16)
Control group (n=13)
Morphological study:
Omalizumab group (n=14)
Control group (n=10)

Figure 2.

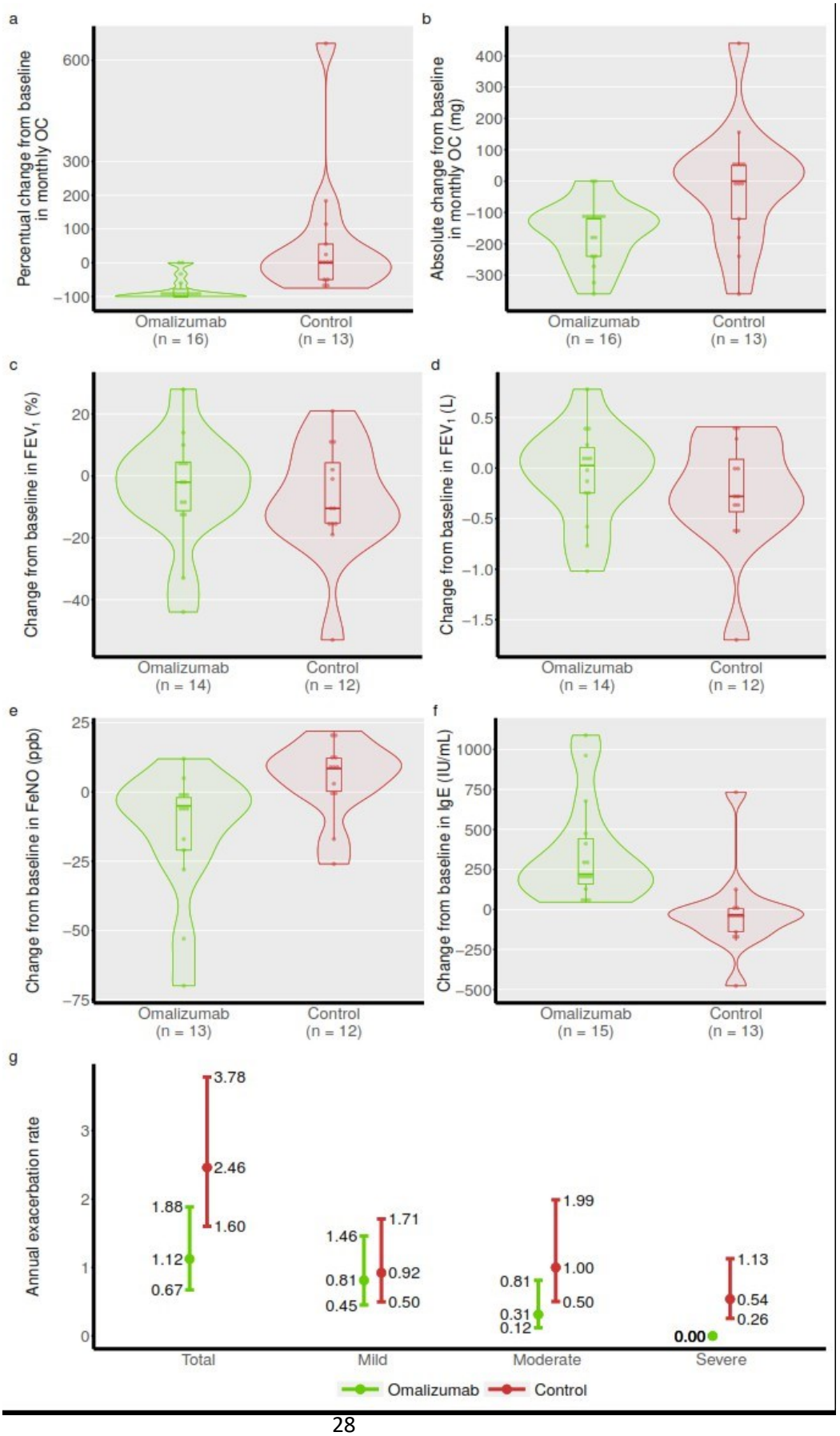

a
Percentual change from baseline in monthly OC
600
300
200
100
0
−100
Omalizumab (n = 16)
Control (n = 13)
b
Absolute change from baseline in monthly OC (mg)
400
300
200
100
0
−100
−200
−300
Omalizumab (n = 16)
Control (n = 13)
c
Change from baseline in FEV1 (%)
20
0
−20
−40
Omalizumab (n = 14)
Control (n = 12)
d
Change from baseline in FEV1 (L)
0.5
0.0
−0.5
−1.0
−1.5
Omalizumab (n = 14)
Control (n = 12)
e
Change from baseline in FeNO (ppb)
25
0
−25
−50
−75
Omalizumab (n = 13)
Control (n = 12)
f
Change from baseline in IgE (IU/mL)
1000
750
500
250
0
−250
−500
Omalizumab (n = 15)
Control (n = 13)
g
Annual exacerbation rate
3
2
1
0
3.78
2.46
1.88
1.60
1.12
0.67
1.71
1.46
0.92
0.81
0.50
0.45
1.99
1.00
0.81
0.50
0.31
0.12
1.13
0.54
0.26
0.00
Total
Mild
Moderate
Severe
Omalizumab
Control

Figure 3.

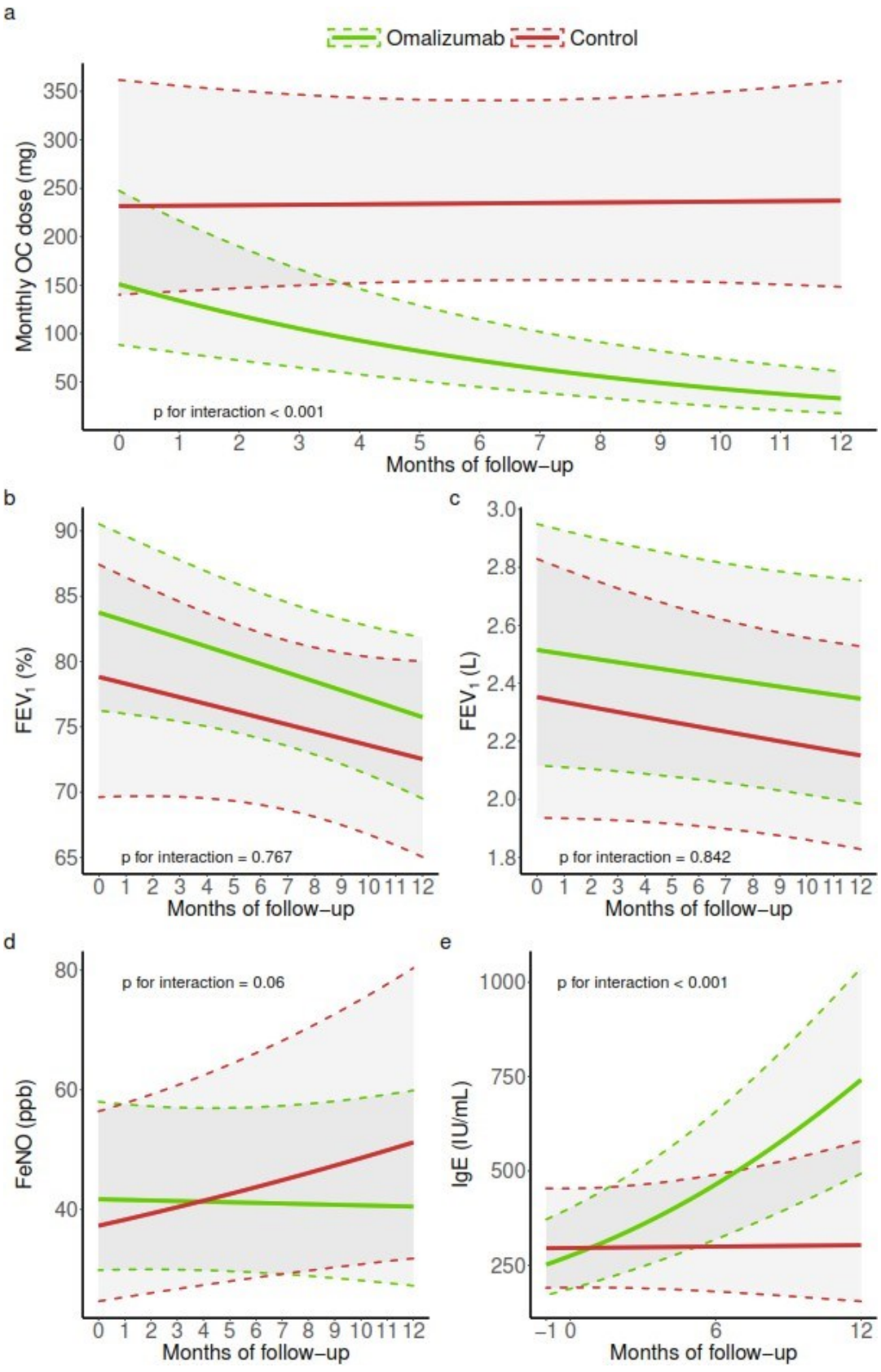

a
Omalizumab
Control
Monthly OC dose (mg)
350
300
250
200
150
100
50
p for interaction < 0.001
0 1 2 3 4 5 6 7 8 9 10 11 12
Months of follow−up
b
FEV1 (%)
90
85
80
75
70
65
p for interaction = 0.767
0 1 2 3 4 5 6 7 8 9 10 11 12
Months of follow−up
c
FEV1 (L)
3.0
2.8
2.6
2.4
2.2
2.0
1.8
p for interaction = 0.842
0 1 2 3 4 5 6 7 8 9 10 11 12
Months of follow−up
d
FeNO (ppb)
80
60
40
p for interaction = 0.06
0 1 2 3 4 5 6 7 8 9 10 11 12
Months of follow−up
e
IgE (IU/mL)
1000
750
500
250
p for interaction < 0.001
−1 0 6 12
Months of follow−up

Figure 4.

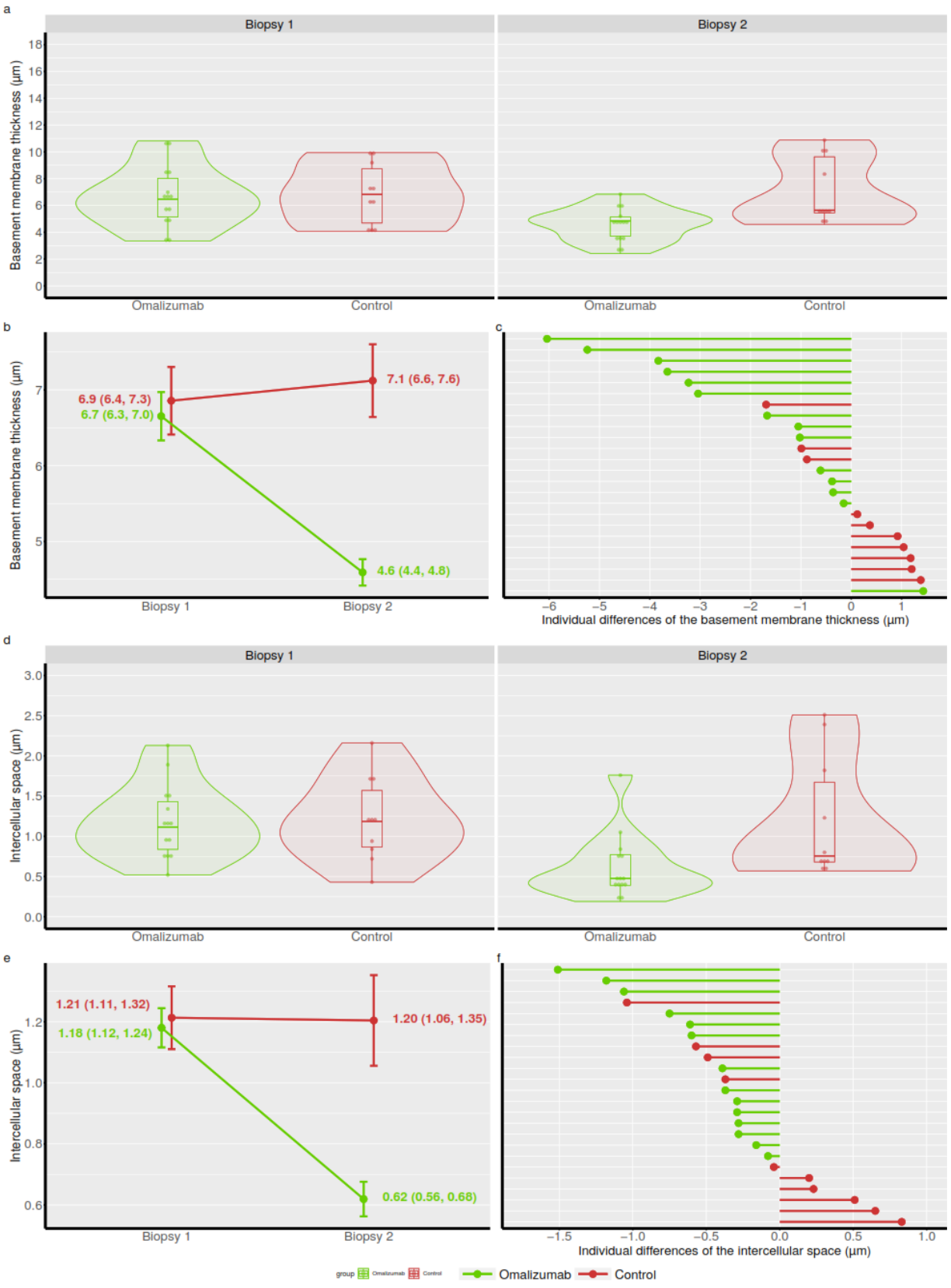
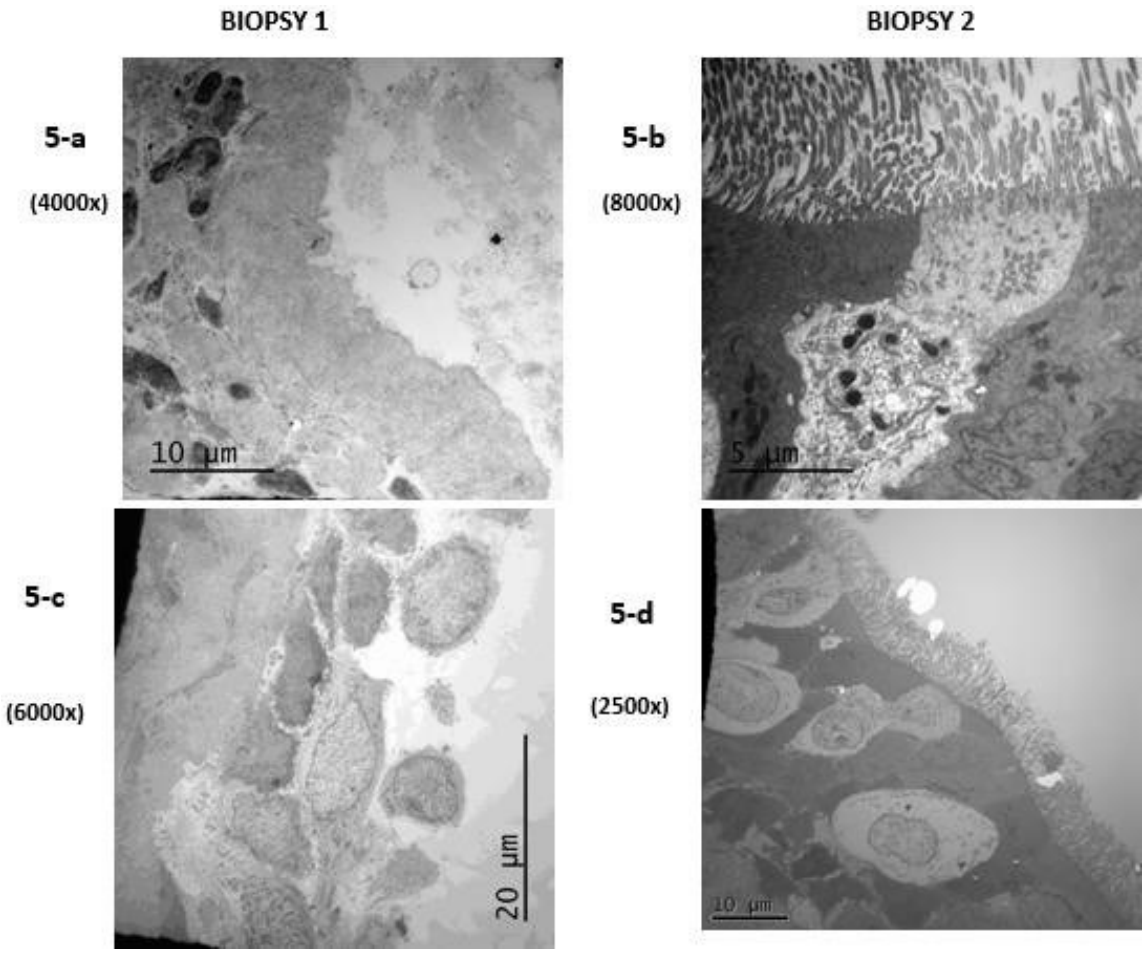

a
Biopsy 1
Biopsy 2
Basement membrane thickness (μm)
Omalizumab
Control
b
Basement membrane thickness (μm)
6.9 (6.4, 7.3)
6.7 (6.3, 7.0)
7.1 (6.6, 7.6)
4.6 (4.4, 4.8)
Biopsy 1
Biopsy 2
c
Individual differences of the basement membrane thickness (μm)
d
Biopsy 1
Biopsy 2
Intercellular space (μm)
Omalizumab
Control
e
Intercellular space (μm)
1.21 (1.11, 1.32)
1.18 (1.12, 1.24)
1.20 (1.06, 1.35)
0.62 (0.56, 0.68)
Biopsy 1
Biopsy 2
f
Individual differences of the intercellular space (μm)
group
Omalizumab
Control

Figure 5.


BIOPSY 1
BIOPSY 2
5-a
(4000x)
10 µm
5-b
(8000x)
5 µm
5-c
(6000x)
20 µm
5-d
(2500x)
10 µm

Declarations:

-Funding.

Grants from SEPAR 845-2009/ FUCAP 2008/FUCAP 2009

-Conflicts of Interest.

CD declares having received financial aid for travel support and speakers bureaus from Novartis, Sanofi, GSK, TEVA, MSD, Esteve, Almirall, Astra-Zeneca, Chiesi, Menarini, Pfizer, Ferrer, Stallergenes, ALK-Abelló, Allergy therapeutics, Hall Allergy, Inmunotek, Roxall.

RMM, CF, and FG declare that they have no conflicts of interest.

No authors have any conflict of interest in relation to this study.

-Availability of data and material.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

-Ethics approval –

The study was approved by the Institutional Review Boards of both hospitals. Written informed consent was obtained from all patients. The study was registered with EudraCT number 2009-010914-31. The study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

-Consent to participate.

"Written informed consent was obtained from all patients"

-Consent for publication Written informed consent was obtained from all patients"

-Code availability. NA

-Authors' contributions:

CD: Conceptualization, funding acquisition, data curation, methodology, project administration, resources, supervision, verification, visualization, writing original draft.

RMM: Conceptualization, data curation, methodology, resources, supervision, verification, visualization, writing original draft.

FJGB: data curation, resources, verification, writing -review & editing.

CF: Formal Analysis, Methodology, Visualization, Writing -review & editing.

FP: data curation, resources, verification, Writing -review & editing.

All authors have read and approve the final version of the manuscript, and agree to be accountable for the work.

-FINANCIAL DISCLOSURE

CD declares having received financial disclosure for travel support and speakers bureaus from Novartis, Sanofi, GSK, TEVA, MSD, Esteve, Almirall, Astra-Zeneca, Chiesi, Menarini, Pfizer, Ferrer, Stallergenes, ALK-Abelló, Allergy therapeutics, Hall Allergy, Inmunotek, Roxall.

FJGB declares having received consulting fees and speakers bureaus from ALK, Astra-Zeneca, Bial, Chiesi, Gebro Pharma, GlaxoSmithKline, Menarini, Novartis, Rovi, Roxall, Sanofi, Stallergenes-Greer, Teva and participated in advisory boards for ALK, Astra-Zeneca, GlaxoSmithKline, Menarini, Novartis, Sanofi, Teva.

RMM, CF, and FG declare that they have no conflicts of interest.

No author declares a conflict of interest in relation to this study.

Figure 1

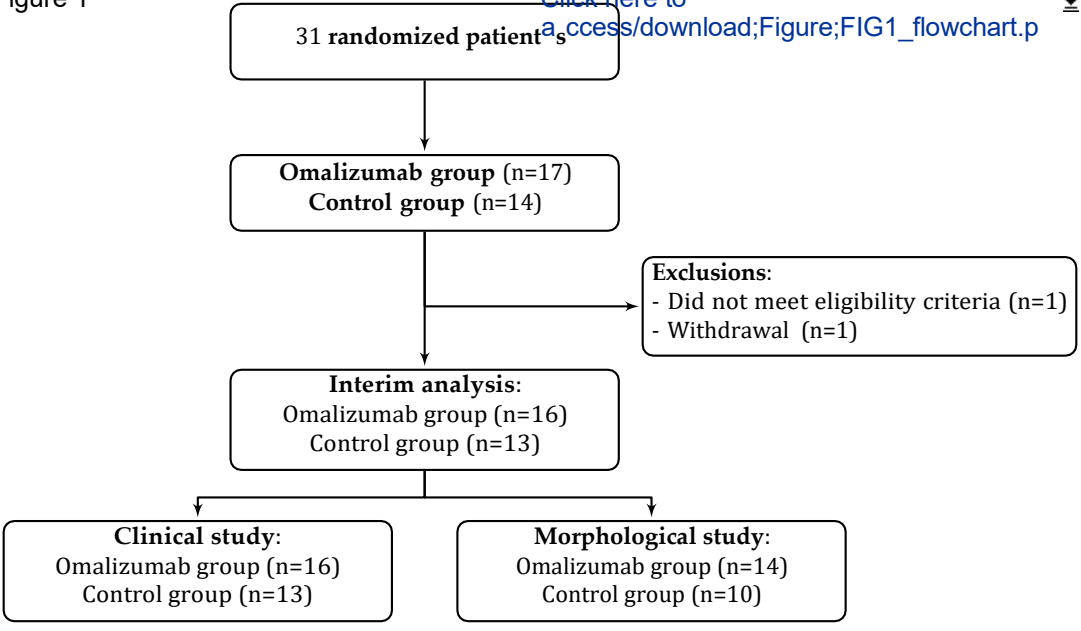
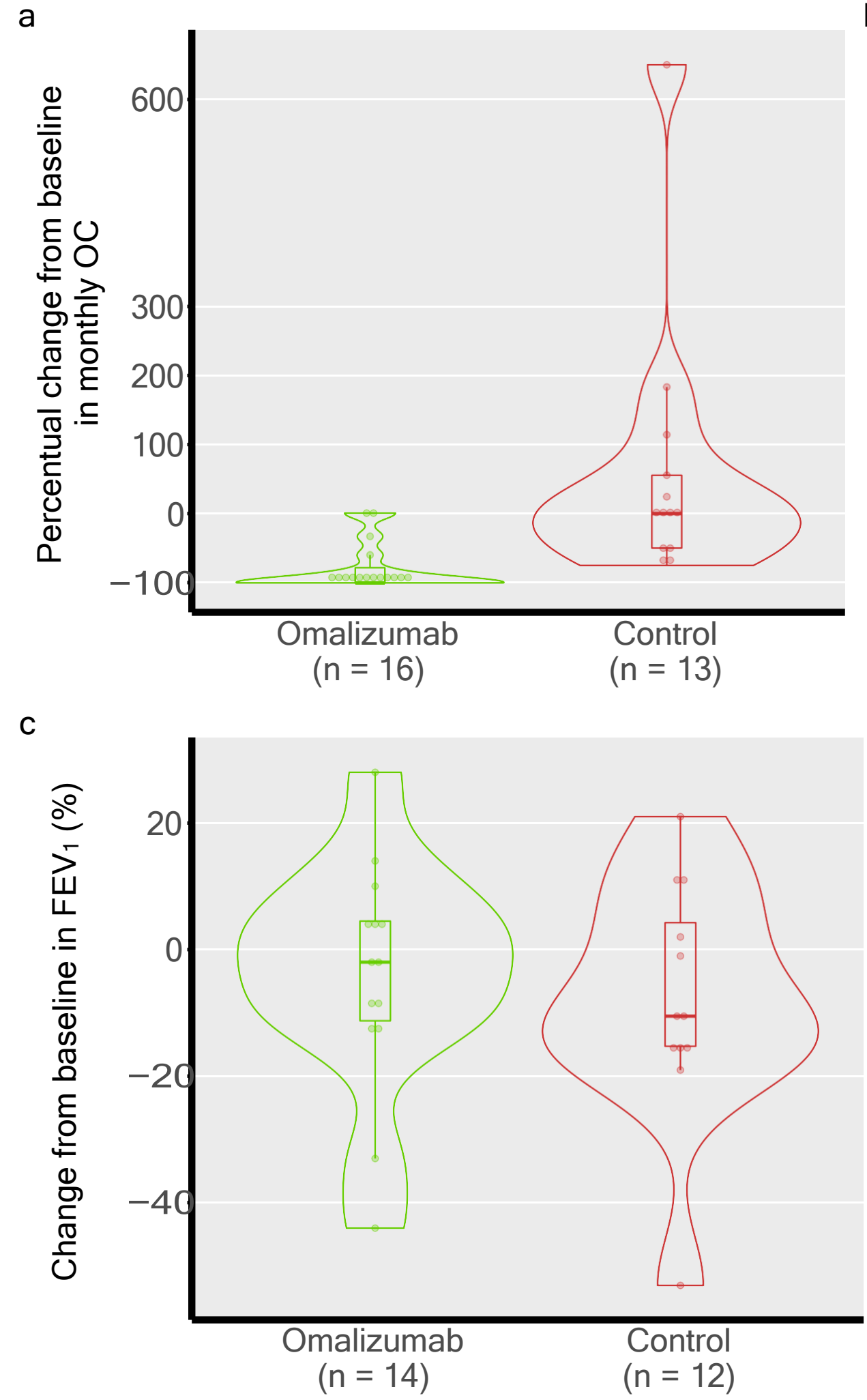
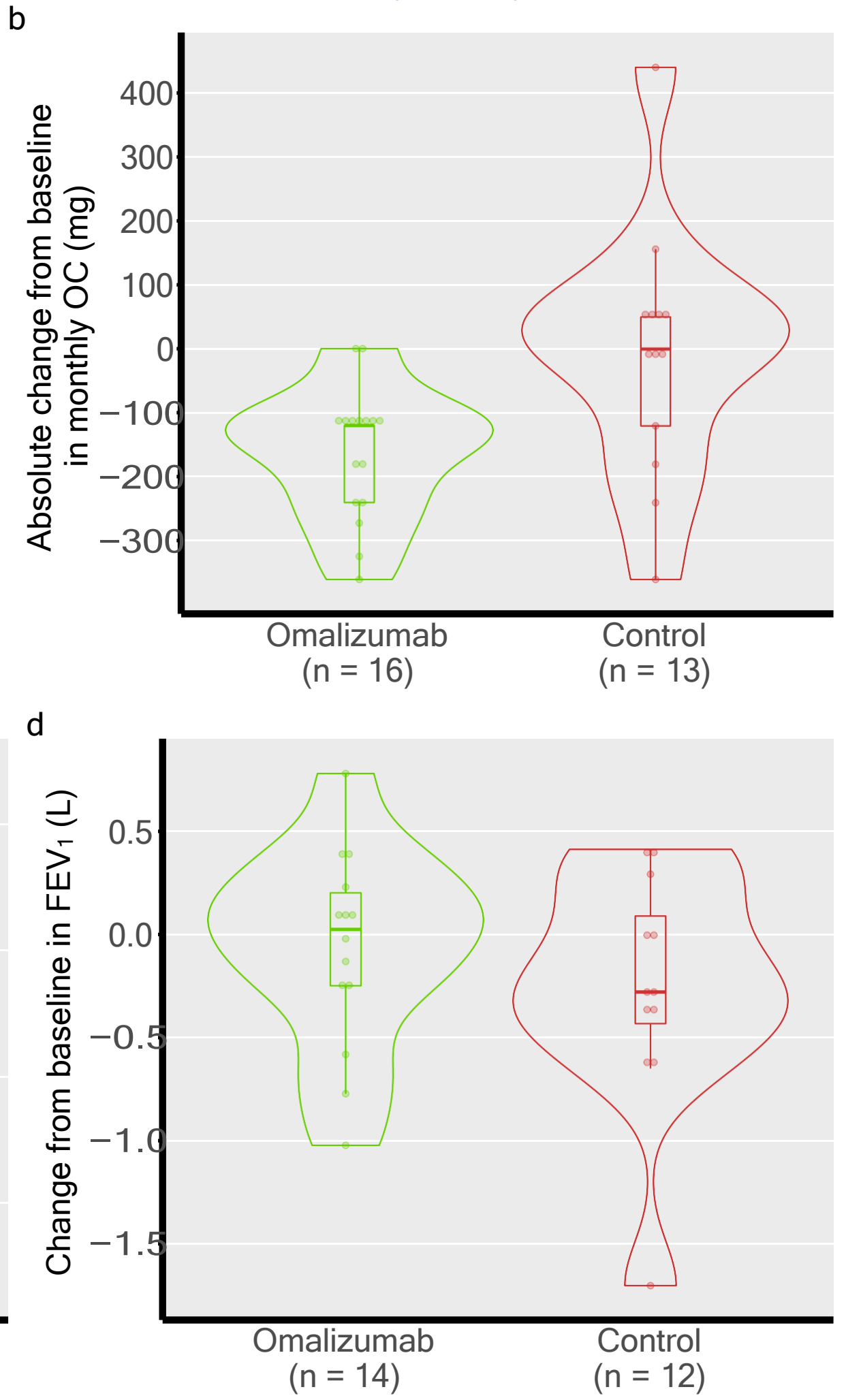
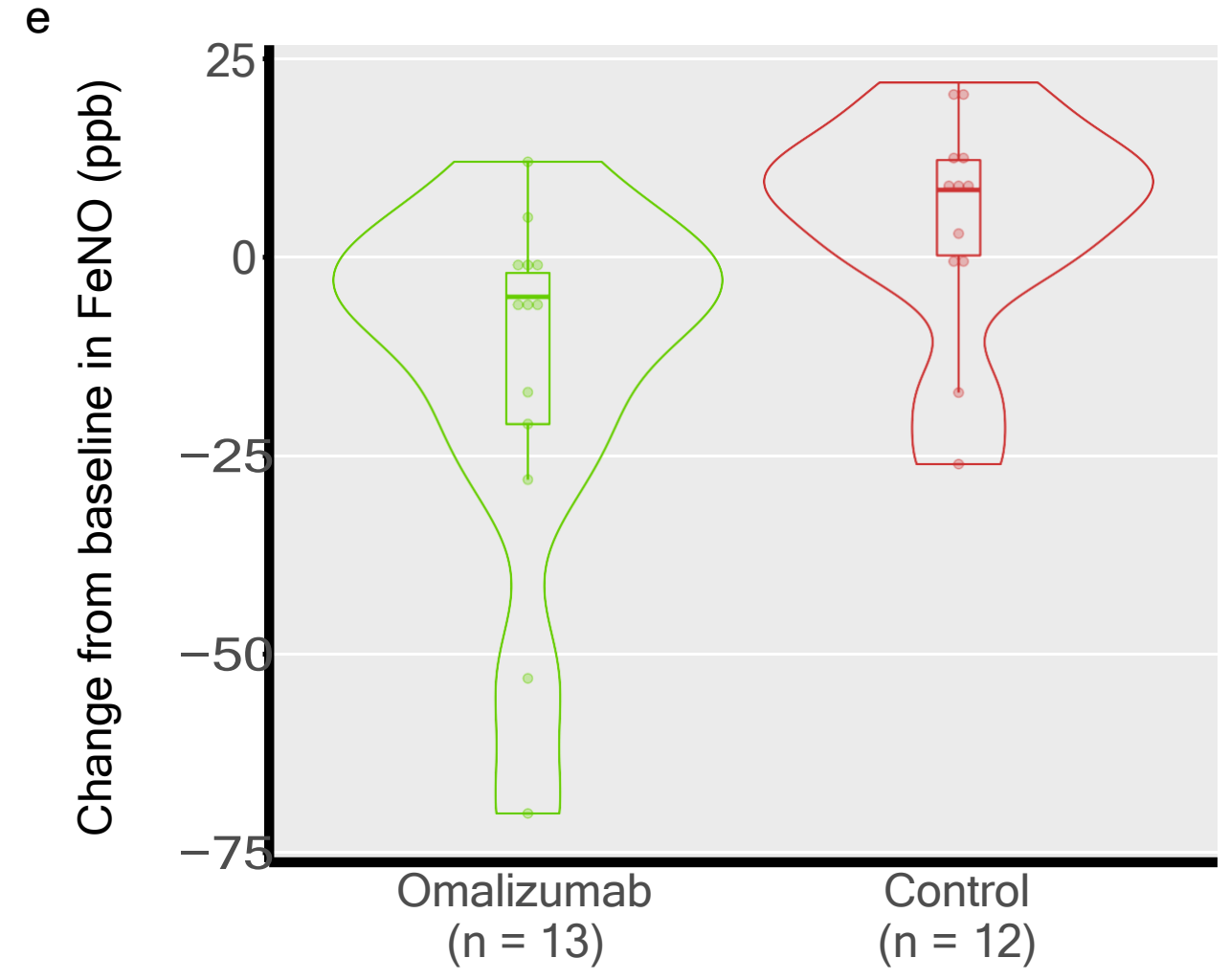
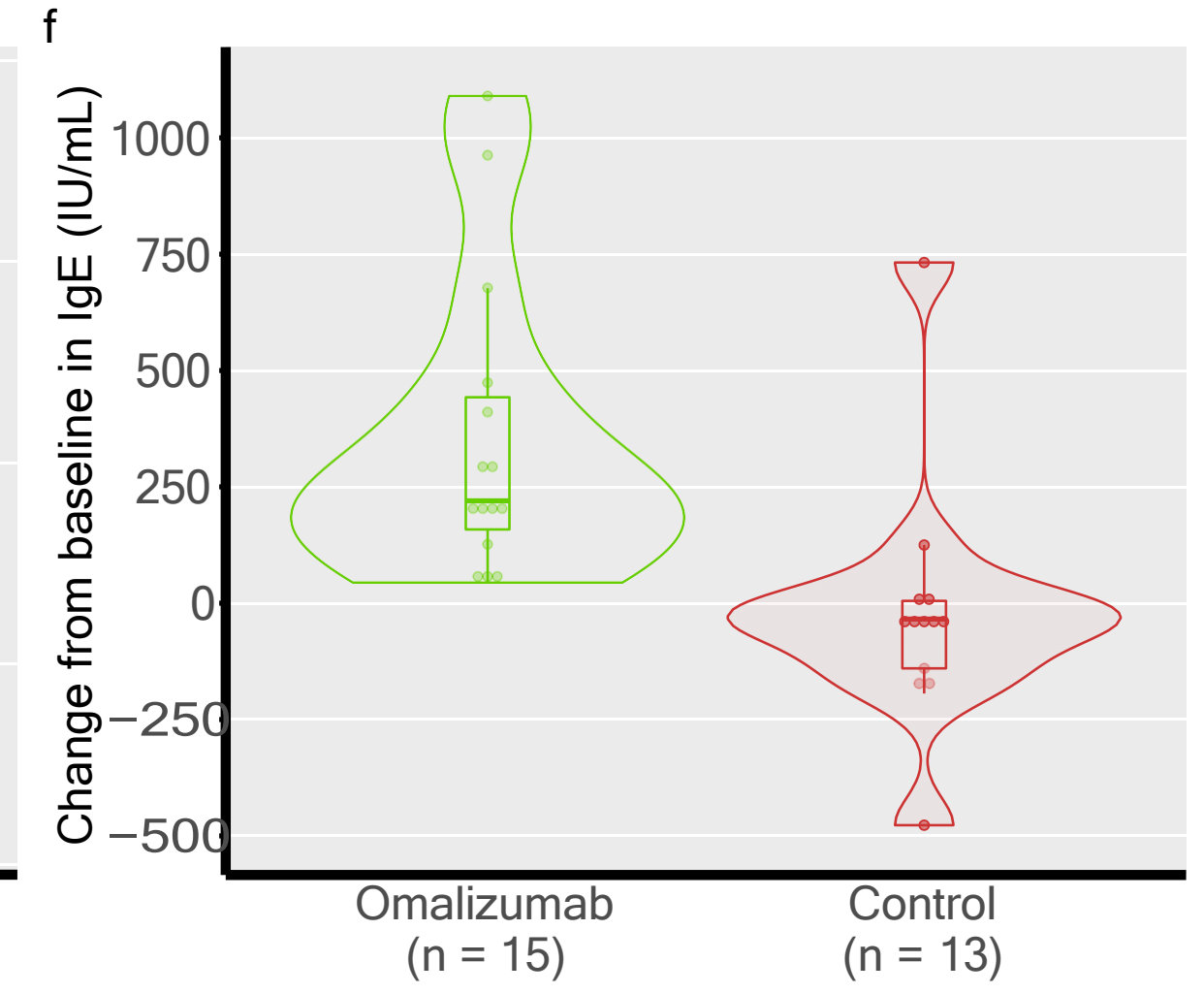


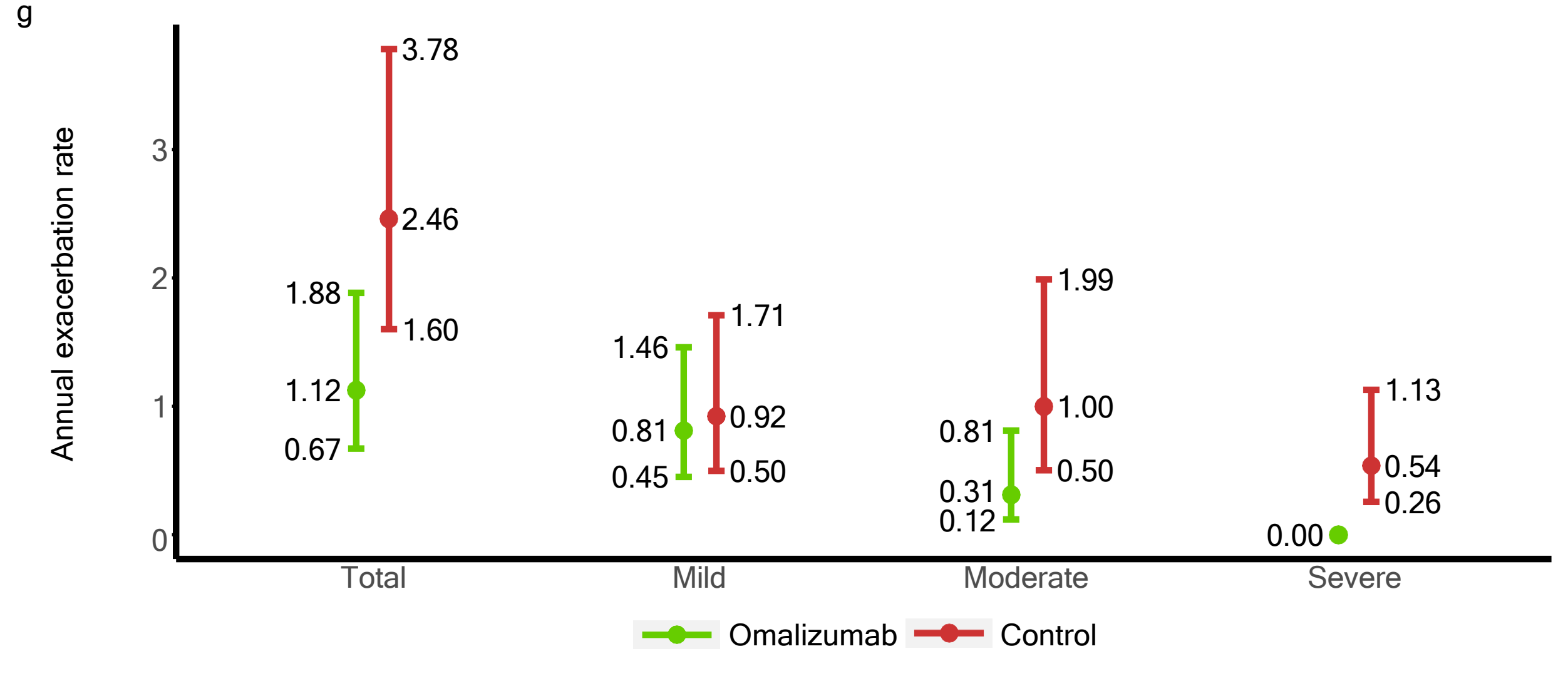
Figure 2



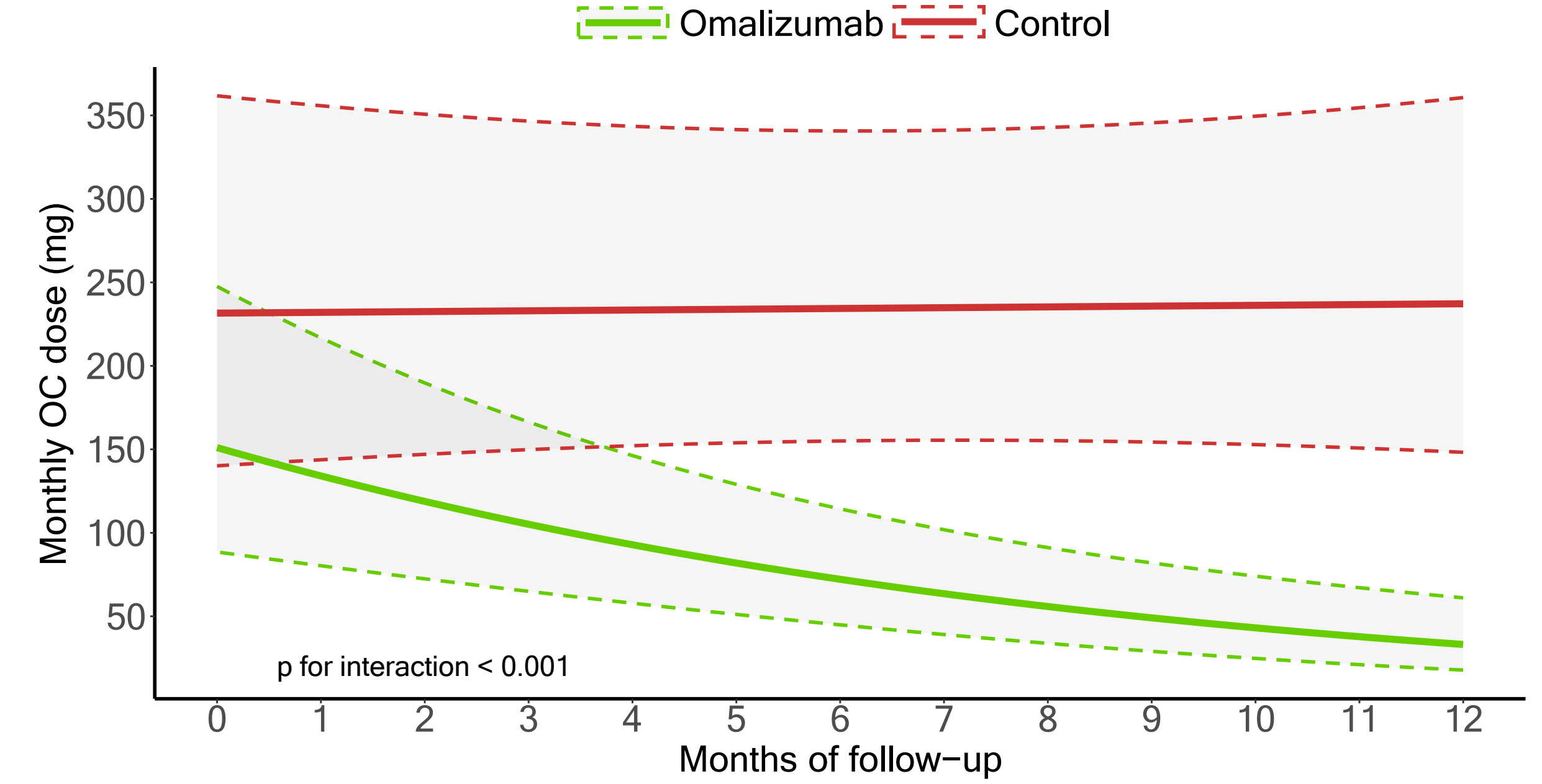




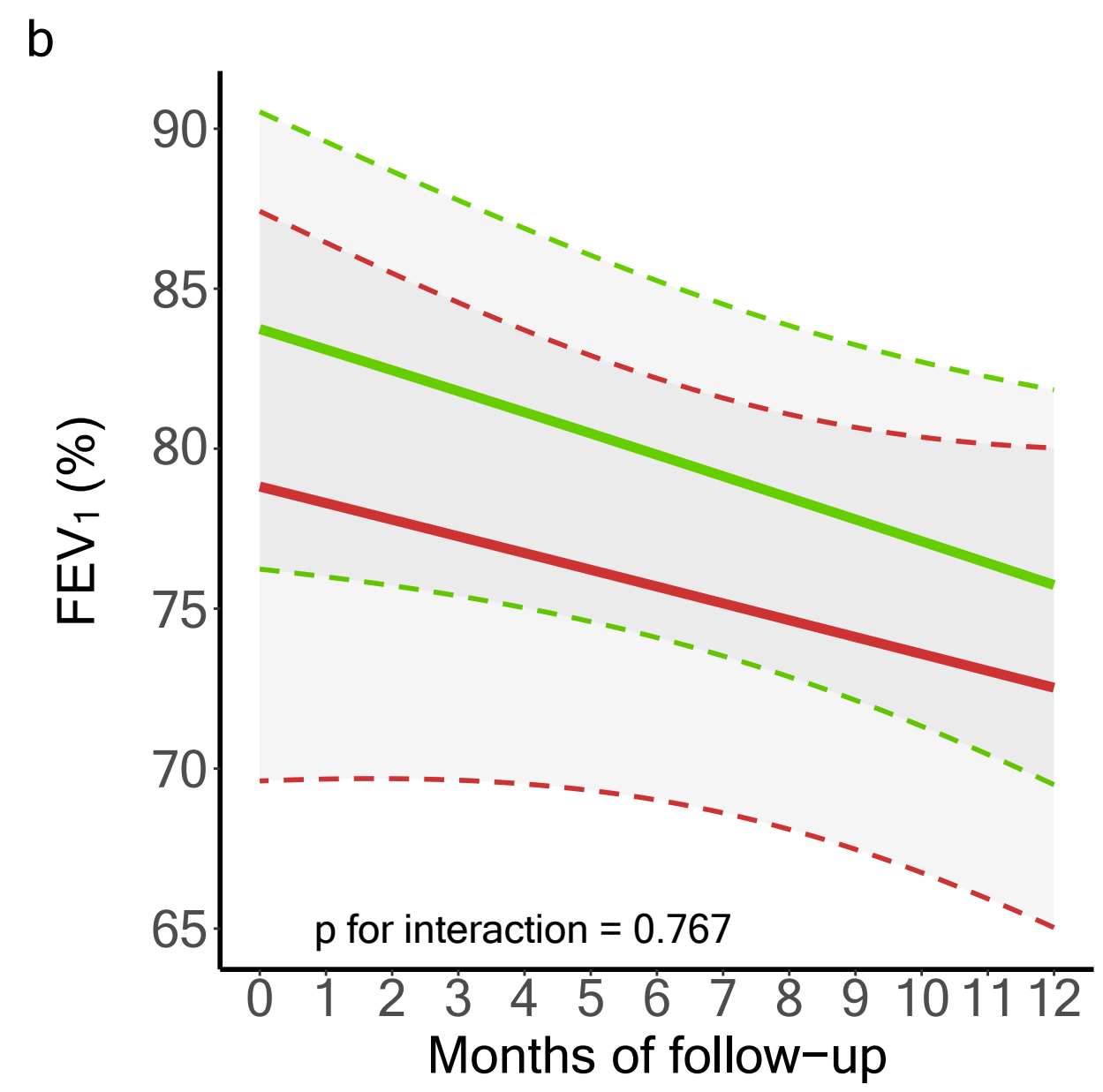




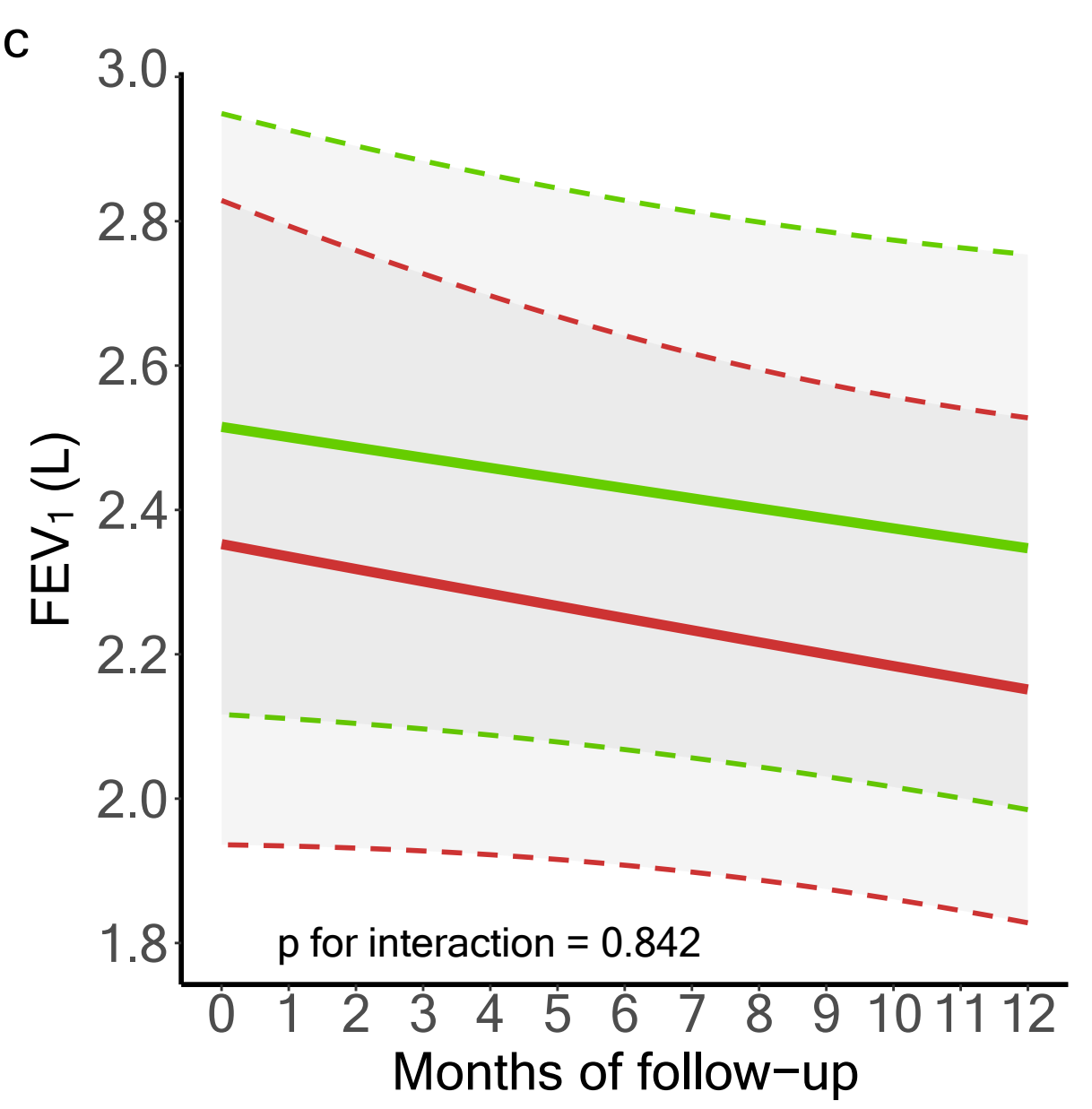
a



b



c



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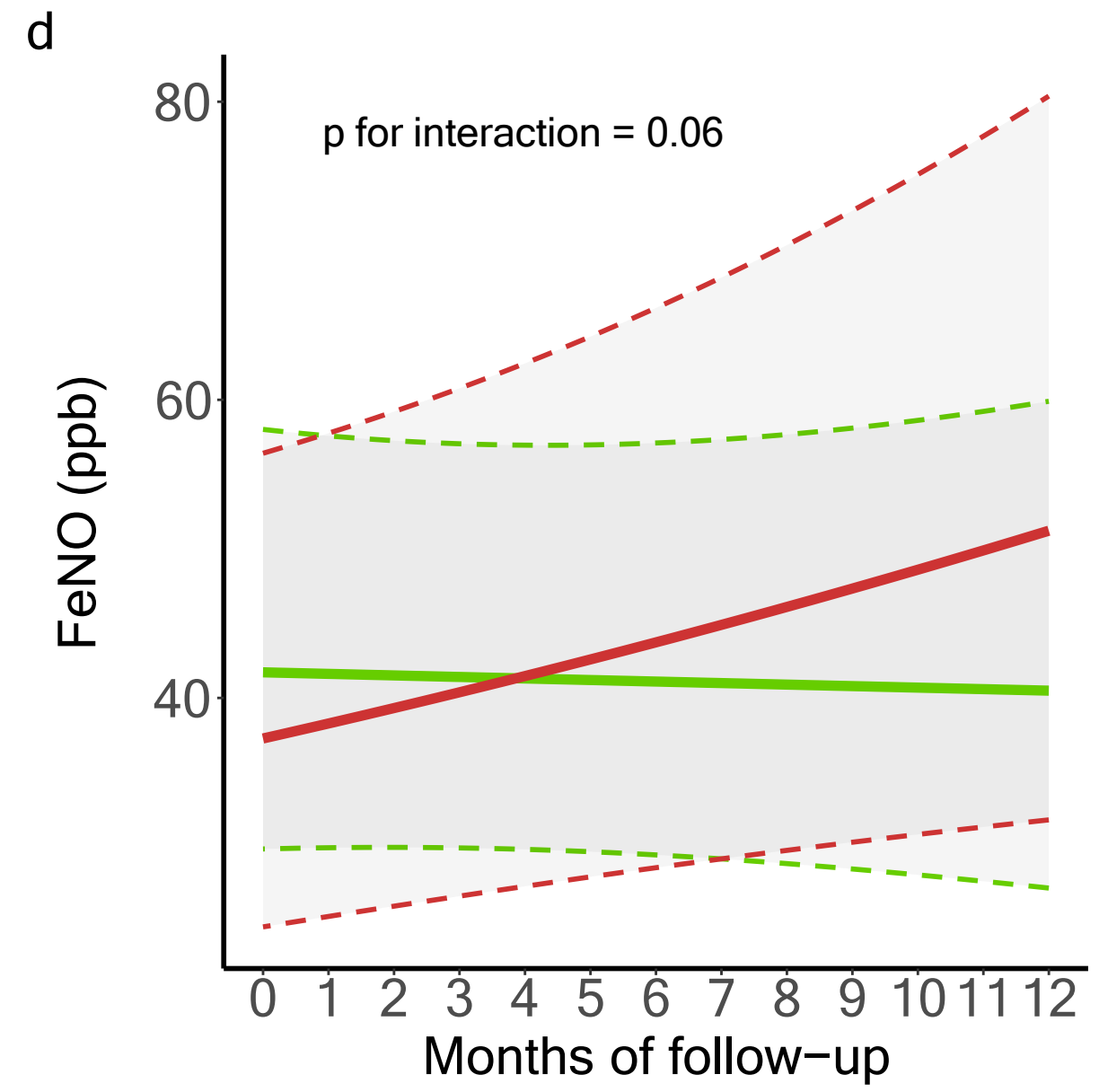


e

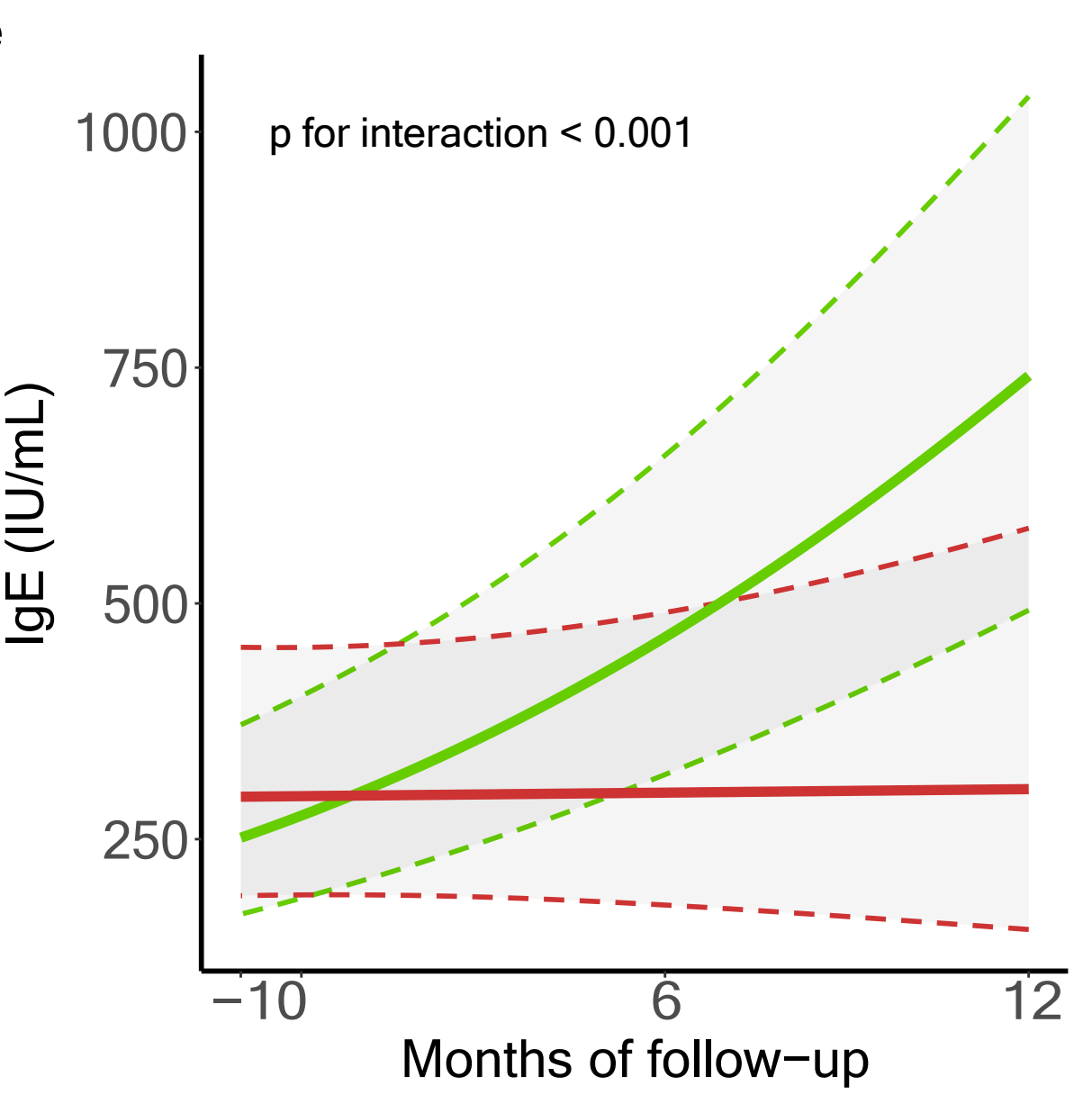
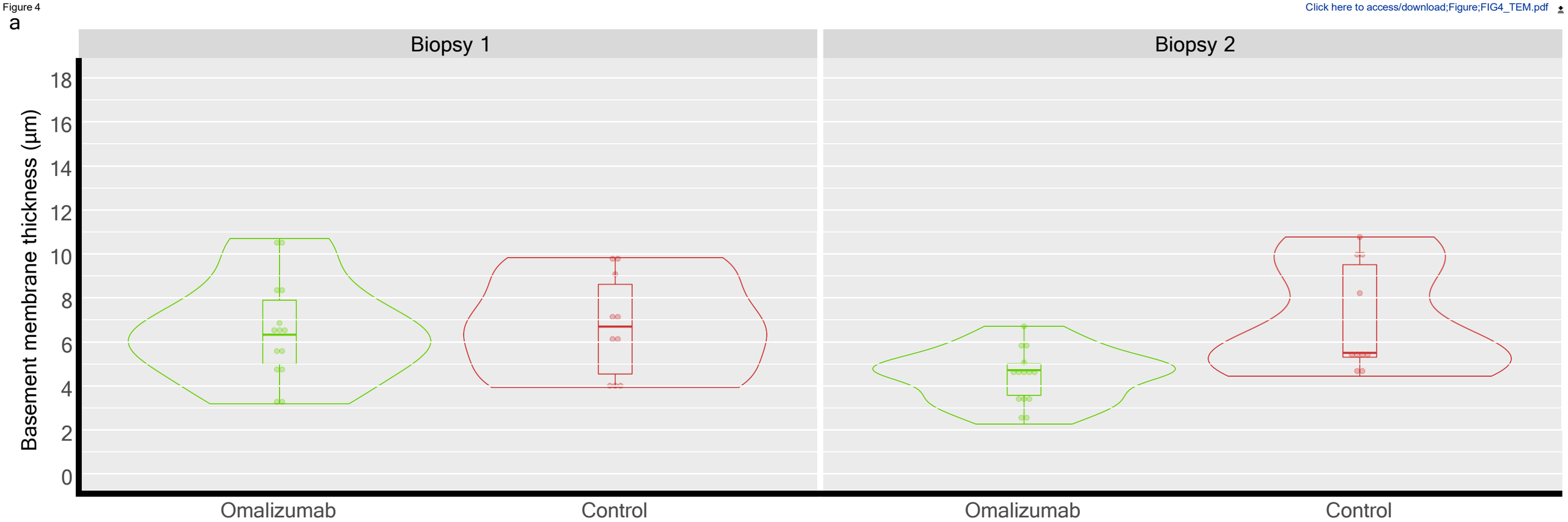
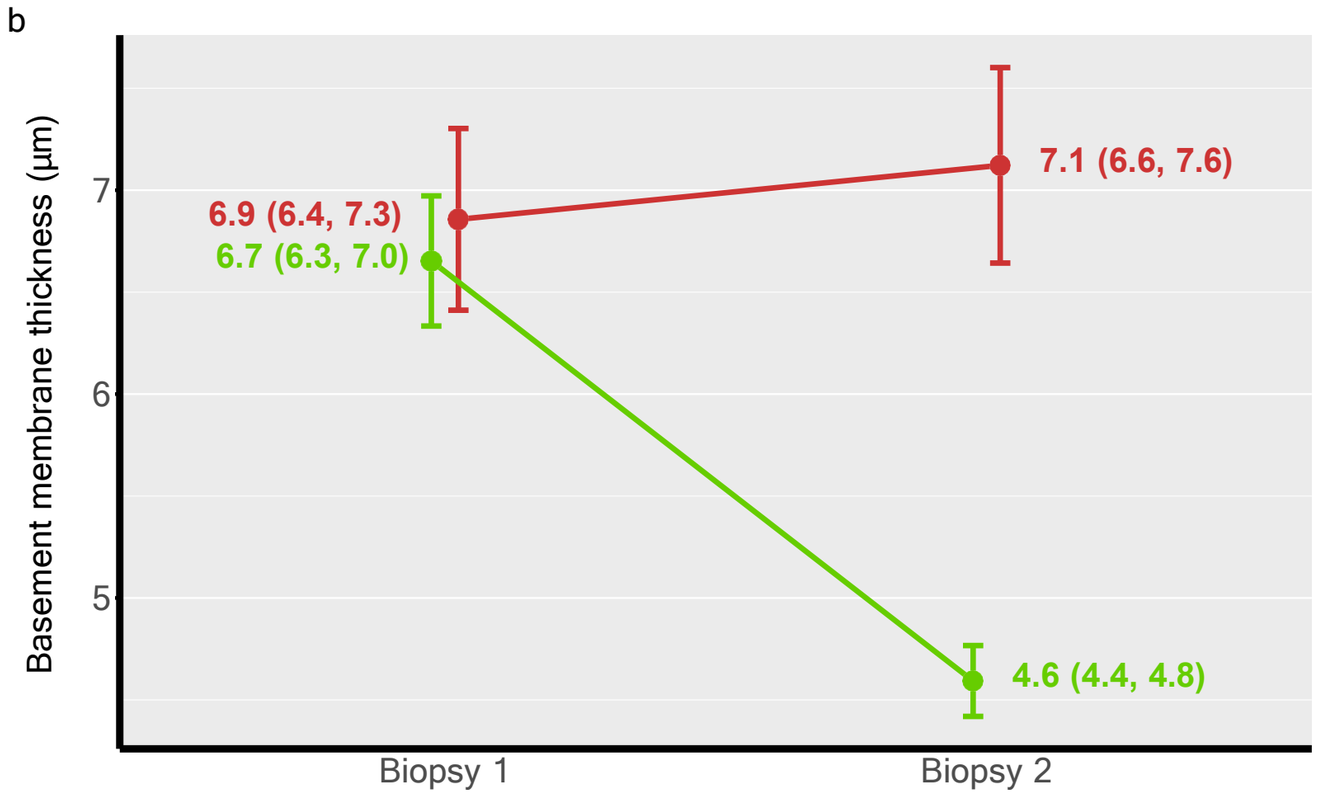
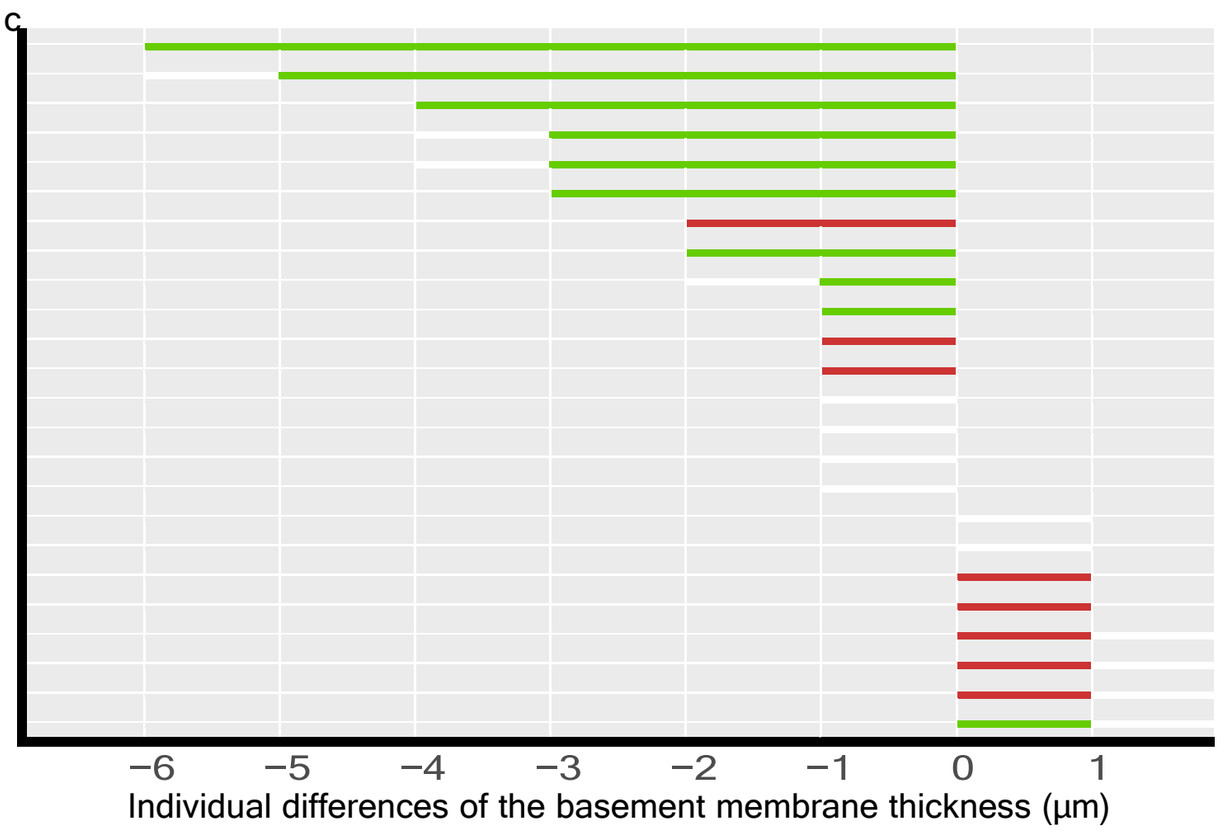
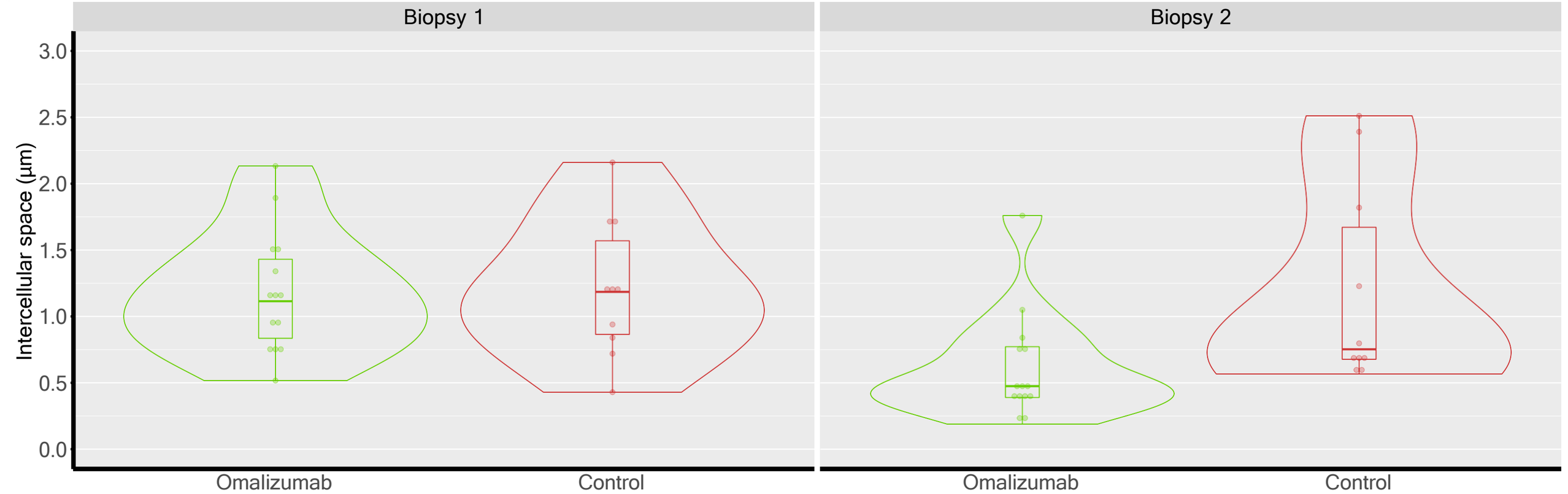


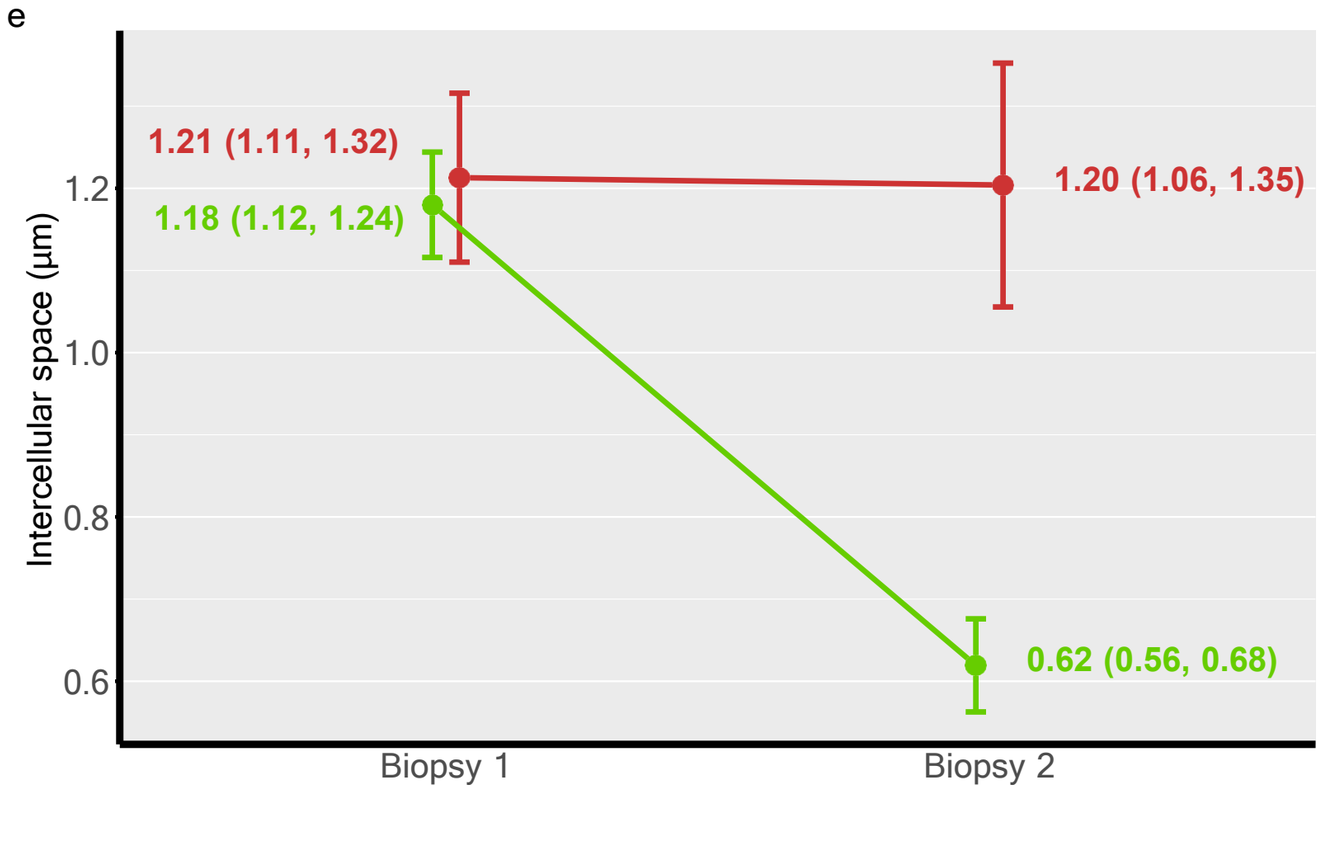
Figure 4











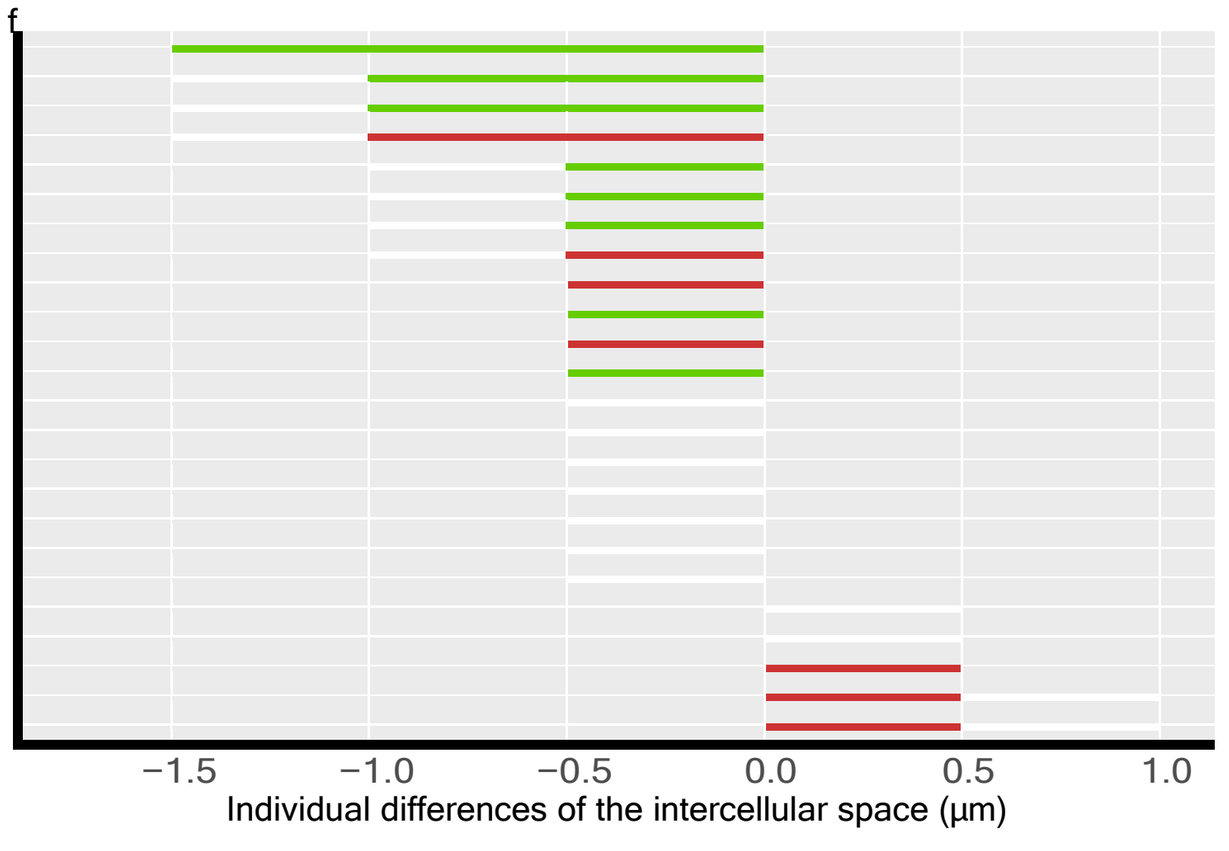


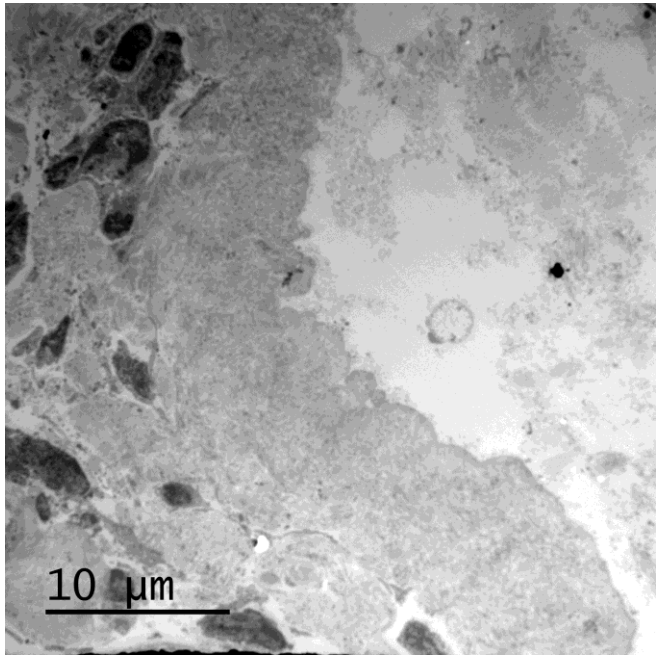
Figure 5

BIOPSY 1

BIOPSY 2

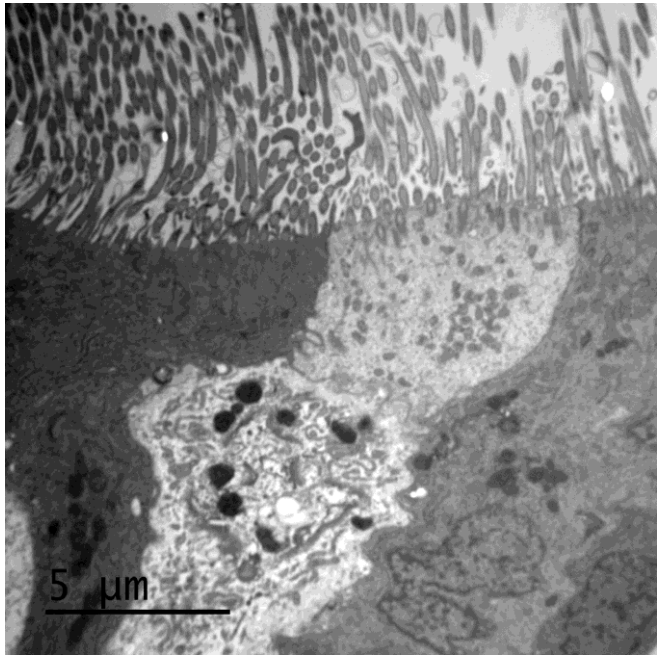
5-a

(4000x)



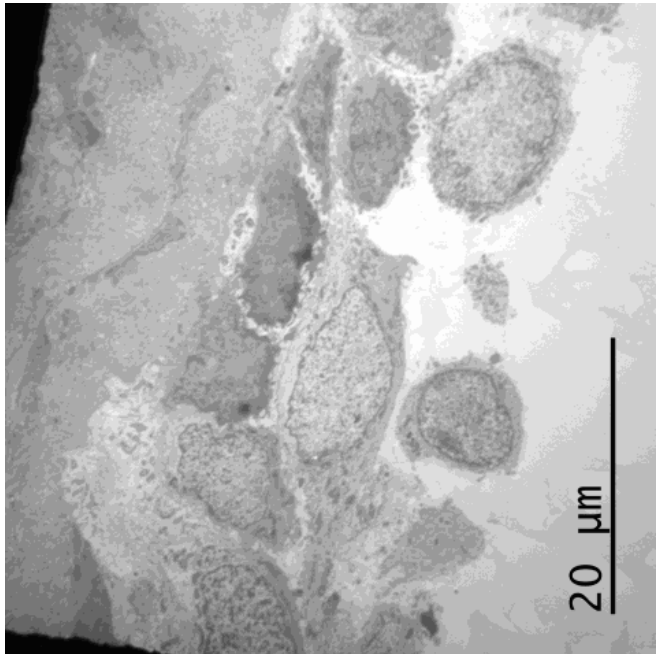
5-b

(8000x)



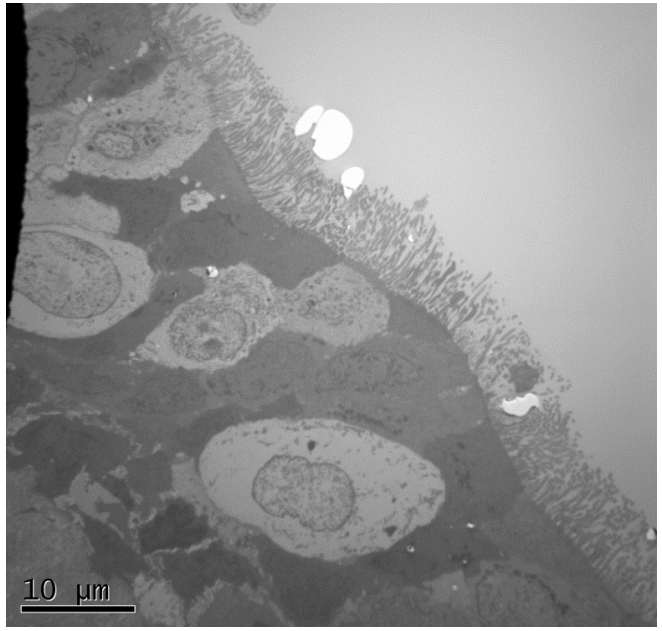
5-c

(6000x)



5-d

(2500x)



DRUGS

Support material for the manuscript:

Clinical and histological impact of omalizumab in allergic asthma patients receiving oral corticosteroids; a randomized open-label parallel study.

By Christian Domingo, Rosa M. Mirapeix, Francisco-Javier González-Barcala, Carles Forné and Felip García.

Index

1.- Inclusion and exclusion criteria

Inclusion criteria: 1) Age ≥18 years. 2) Bronchial asthma, defined as a spontaneous or pharmacologically induced increase of 12% or more and a minimum of 200 ml in the forced expiratory volume in the first second (FEV_1). 3) OC-dependence, defined as a requirement of at least 7.5 mg per day (or 15 mg on every other day) of prednisolone or 6 mg per day (or 12 mg on every other day) of 6-methylprednisolone (6-MP) to maintain an $FEV_1 \geq 70\%$ during a period of one year or more; in case of repeated intermittent OC intake, accumulated total of OC dose in 1 year ≥ 1.200 mg. 4) Failure of full-tapering of corticosteroids for at least three months. 5) Allergic asthma, defined by a positive skin prick test and/or an immuneCAP specific IgE test + allergic symptoms. 6) IgE level between 30 and 1500 IU/mL. 7) Body weight < 150 Kg. 8) Signed written informed consent.

Exclusion criteria: 1) Prior exposure or sensitivity to omalizumab. 2) Acute upper respiratory tract infection in the last month. 3) Patients receiving specific immunotherapy. 4) Elevated IgE levels for reasons other than atopy. 5) Requirement of omalizumab doses > 1200 mg per 4 weeks. 6) Refusal to sign the informed consent before enrolment.

2.- Study protocol

Period	1	2	3												
Day	-120 to -30	-29 to 0	0	30	60	90	120	150	180	210	240	270	300	330	360
Month	-4 to -1	-1 to 0	0	1	2	3	4	5	6	7	8	9	10	11	12
Visit	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12
Informed consent	X														
Inclusion/exclusion criteria	X	X													
CTS adjustment	X	X													
Skin Prick Test[1]	X														
Body weight	X	X													
Treatment randomization	X														
Measurement of FeNO			X	X	X	X	X	X	X	X	X	X	X	X	X
Blood analysis	X								X						X
Total IgE	X								X						X
Bronchoscopy/Bronchial biopsy			X												X
Exacerbations	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Forced spirometry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AAs		X		X	X	X	X	X	X	X	X	X	X	X	X
Daily OC dose	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Accumulated OC dose/month			X	X	X	X	X	X	X	X	X	X	X	X	X

Prick Test[1] In the presence of dermatography, an immunoCAP analysis was performed. The Prick test was only performed if the patient had not undergone this test in the past year

3.- Skin prick test

Skin prick tests were carried out using a standardized allergen extract (SoluPrick®; ALK, Copenhagen, Denmark) against the following allergens: birch, timothy grass (*Phleum pratense*), mugwort (*Artemisia vulgaris*), cat, dog, horse, house dust mite (*Dermatophagoides pteronyssinus and farinae*), *Cladosporium*, and *Alternaria*.

4.- Bronchoscopy technique

- All subjects received 2.5 mg nebulized salbutamol and 0.5 mg of atropine via intramuscular administration as premedication.
- 2% lidocaine was instilled into the oropharynx.
- During the bronchoscopic examination patients were monitored by pulse oximetry (Datex Ohmeda 3.800®).
- 4% lidocaine was instilled via the bronchoscope to the vocal cords and 2% lidocaine was instilled intrabronchially.
- When required for general sedation, 2.5-5 mg midazolam were given intravenously.
- Bronchial biopsy specimens were obtained using an Olympus BF type 160 connected to a video Olympus Exera CV-145 and an Olympus BD21® -cup forceps.

5.- TEM biopsy processing

The biopsies were fixed immediately in 5% glutaraldehyde in cacodylate buffer at pH 7.2. After several changes of buffer, the tissue was post-fixed with 1% osmium tetroxide in cacodylate buffer for 90 min at room temperature, dehydrated in graded series of ethanol, and finally embedded in epoxy resin. Sections 1 μm thick were cut and stained with toluidine blue to determine the orientation of the biopsy by light microscopy. Ultrathin sections (40-60 nm) were stained with uranyl acetate and lead citrate on 300 mesh copper grids for electron microscopic analysis (JEM 1400). Each biopsy had a mean of three-four copper grids and each copper grid had at least nine sections.

To avoid observer bias, all microscopic slides and electron micrographs were coded prior to analysis and read blindly. The observer read all biopsies twice; the comparison of the two measurements did not show statistically significant differences between the scores of qualitative parameters. For the quantitative measurements mean values were accepted.

The quantitative measurements included the thickness of the basement membrane (BM) and the widening of the intercellular spaces. The thickness of the BM was measured in randomly selected sections of at least 30 points (the number of sections ranged between 25-35) at 20 μm intervals in each sample in accordance with the method developed by Sullivan et al (11). The measurements of the intercellular spaces were obtained by transecting a perpendicular line to opposing cell membranes. At least 30 measures, at a magnification of 8000x, were taken randomly from each area (12).

The qualitative evaluation included the description of the epithelium and the cilia. To quantify the degree of damage to the epithelium a scale of 0-4 was used, where 0- no damage, 1- presence of intercellular spaces, 2- detachment of a few columnar cells, 3- detachment of many cells but no basal cells, and 4- total loss of the columnar epithelium. The degree of damage was also classified as A- focal or B- extensive (See more details in the ESM).

Cilia characteristics were studied in all biopsies. Cell surfaces and cilia were studied at a magnification of 1000x and 2500x and details of cilia and microvilli were

studied at a magnification from 40000x to 100000x. The presence of cilia in the epithelium was classified into three categories: 1- normal, 2- focal absence, and 3- extensive absence (See more details in the ESM).

6.- TEM - Quantitative parameters evaluation

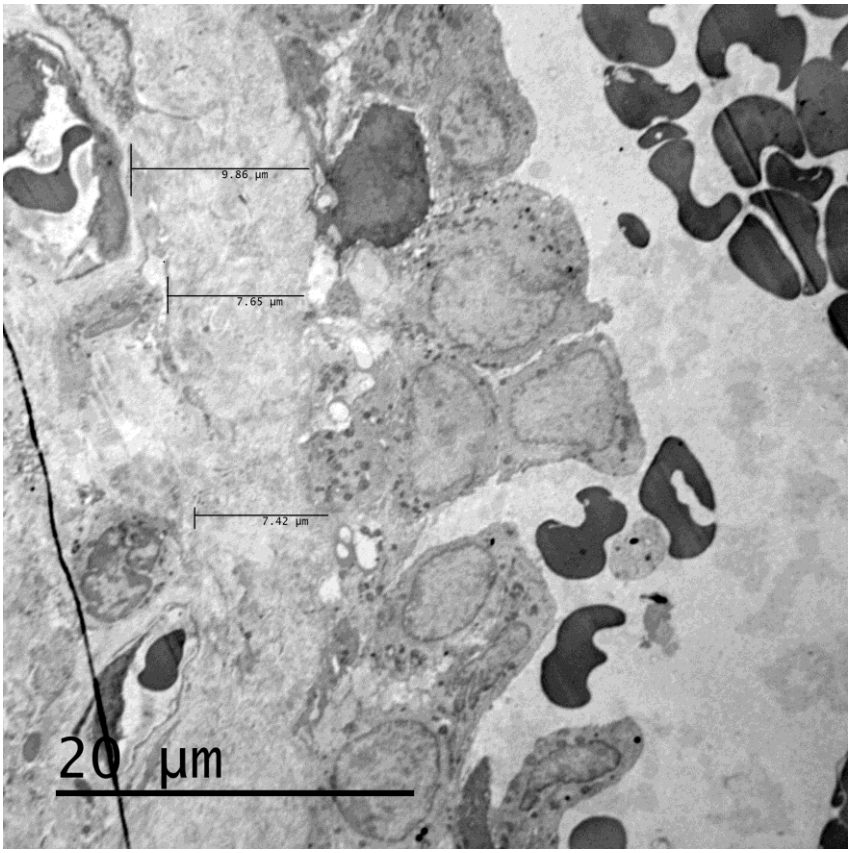
<u>Basement membrane thickness</u> (µm) ***(11)***

- randomly every 7-10 µm

- the number of measurements ranged 25-35

<u>Intercellular space</u> (µm) ***(12)***

- 30 measurements from each area



References in the body text:

11) Sullivan P, Stepens D, Ansari T, Costell J, Jeffery P. Variation in the measurements of basement membrane thickness and inflammatory cell number in bronchial biopsies. European Respiratory Journal 1998; 12:811-815.

12) Johannessen R, Skogaker N, Halgunset J, Petersen H, Kleveland PM. A standardized method for measuring intercellular spaces in esophageal biopsies in patients with suspected gastroesophageal reflux disease (the intercellular space ratio). Scandinavian Journal of Gastroenterology 2013; 48(11):1235-41.

7.- TEM - Qualitative parameters evaluation: epithelium

Epithelium characteristics

5 degrees:

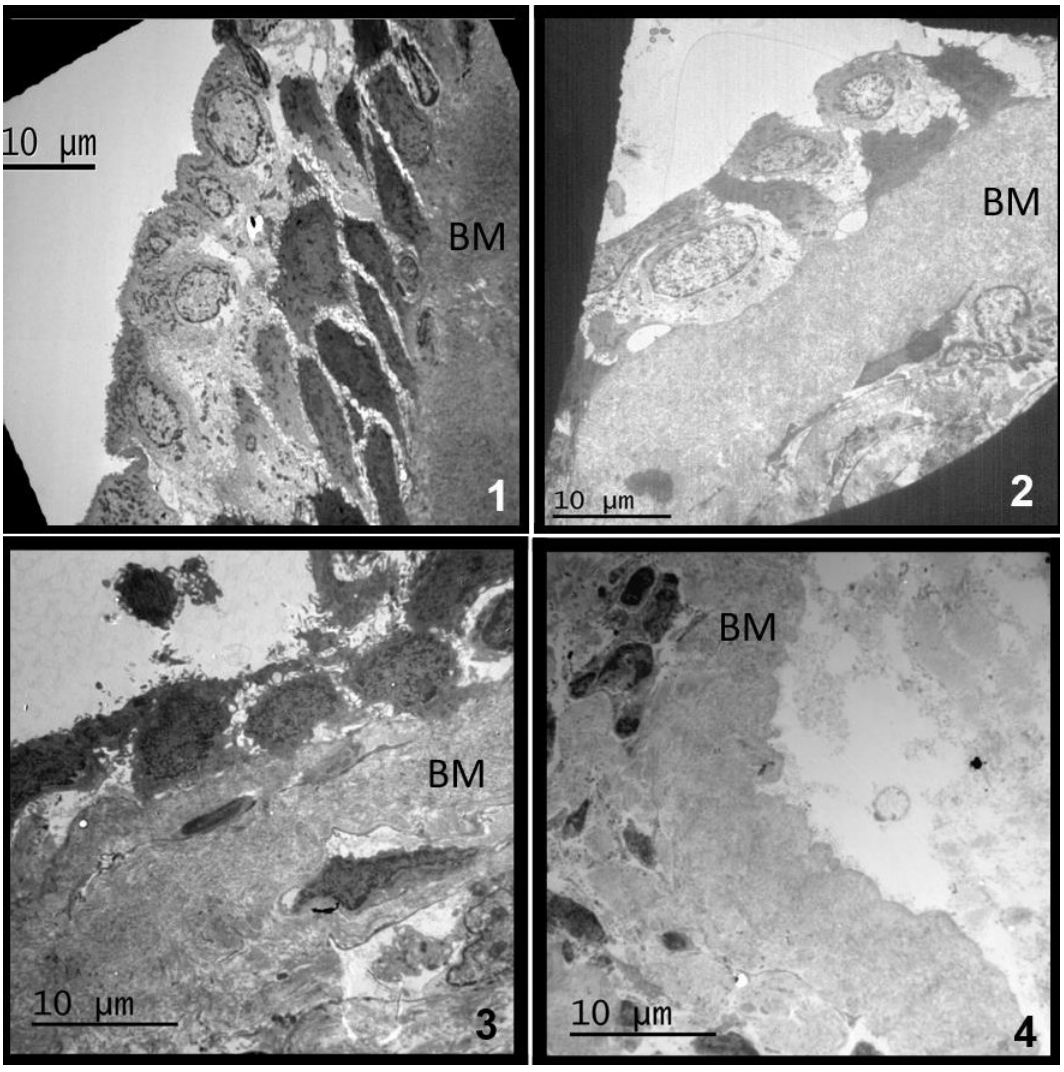
0. No damage
1. Increment of intercellular space without detachment
2. Detachment of a few columnar cells
3. Detachment of most of the epithelium (presence only of basal cells)
4. Total loss of columnar epithelium

2 levels:

a) Focal

b) Extensive

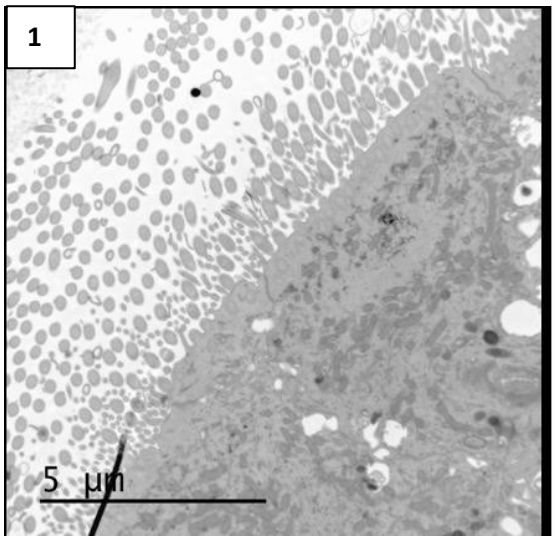
See examples of the 4 degrees of damage (numbered from 1-4) (*Magnification, 2.500 x*)



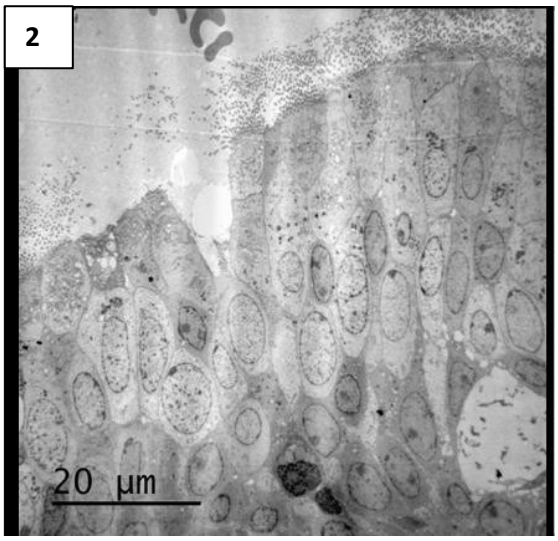
8.- TEM - Qualitative parameters evaluation: cilia

<u>Ciliary study</u>

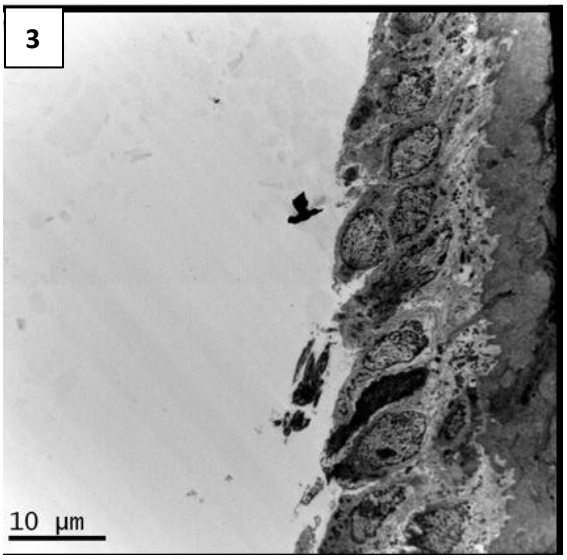
1. Normal presence
2. Focal absence
3. Extensive absence



Magnification, 1200 x



Magnification, 2000 x



Magnification, 2500 x

9.- Table 1s. Details of the test boundaries for interim analysis using the Pocock spending function.

% of recruitment	Lower boundary	Upper boundary	Inc alpha	Total alpha	Total power
50%	-2.157	2.157	0.031	0.031	0.484
100%	-2.201	2.201	0.019	0.050	0.807

Drift: 2.99333

Lower and Upper boundary: These are the test boundaries. If the computed value of the test statistic z is between these values, the trial should continue. Otherwise, the trial can be stopped.

Inc alpha: This is the amount of alpha that is *spent* by this interim test. It is close to, but not equal to, the value of alpha that would be achieved if only a single test was conducted. The difference is due to the correction that must be made for multiple tests.

Total alpha: This is the total amount of alpha that is used up to and including the current test.

Total power: These are the cumulative power values. They are also the cumulative exit probabilities. That is, they are the probability that the trial is stopped at or before the corresponding time.

Drift: This is the value of the Brownian motion drift parameter.

10.- Figures

Figure 1s. Oral corticosteroid consumption

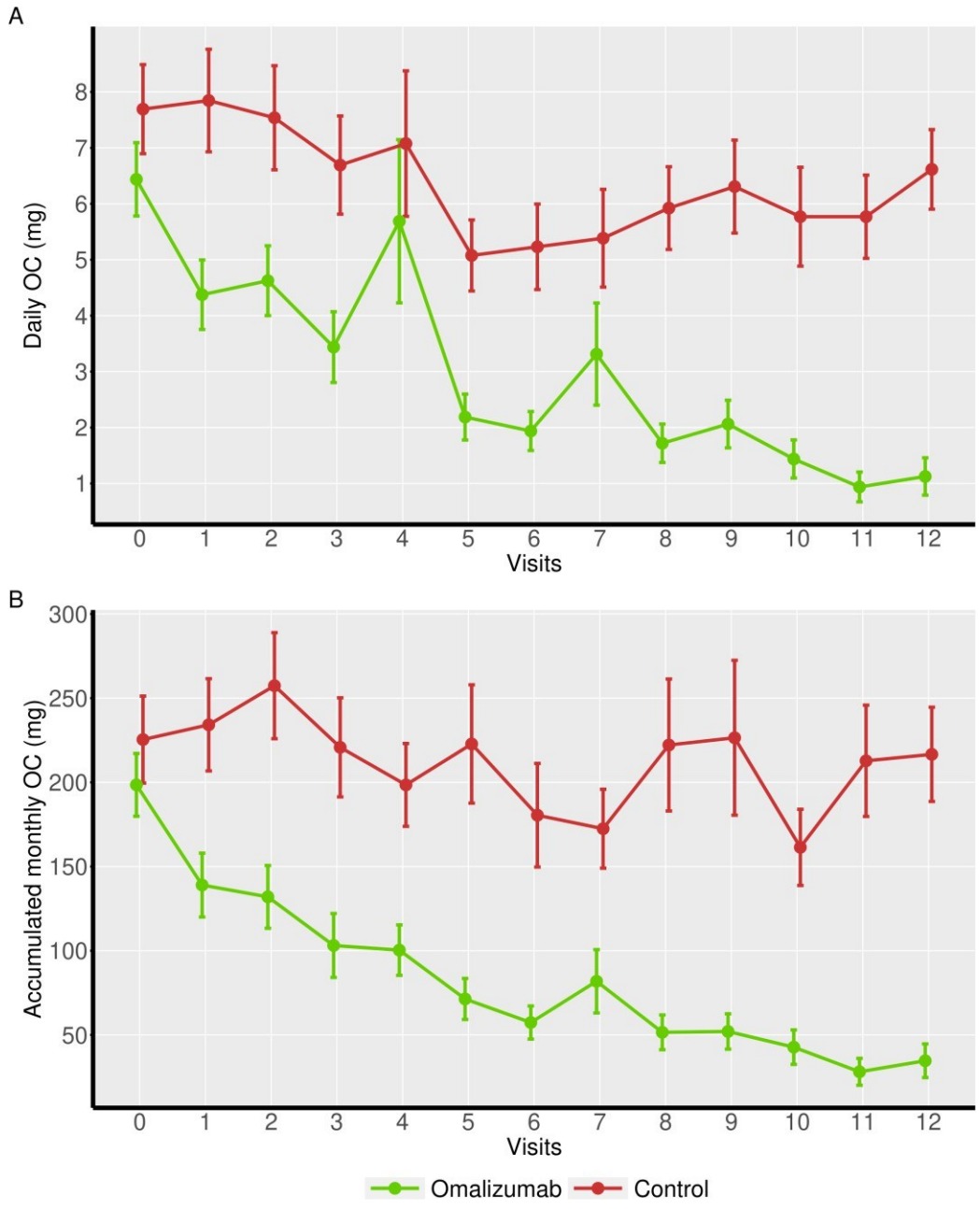


Figure 2s. Monthly changes of pulmonary function testing

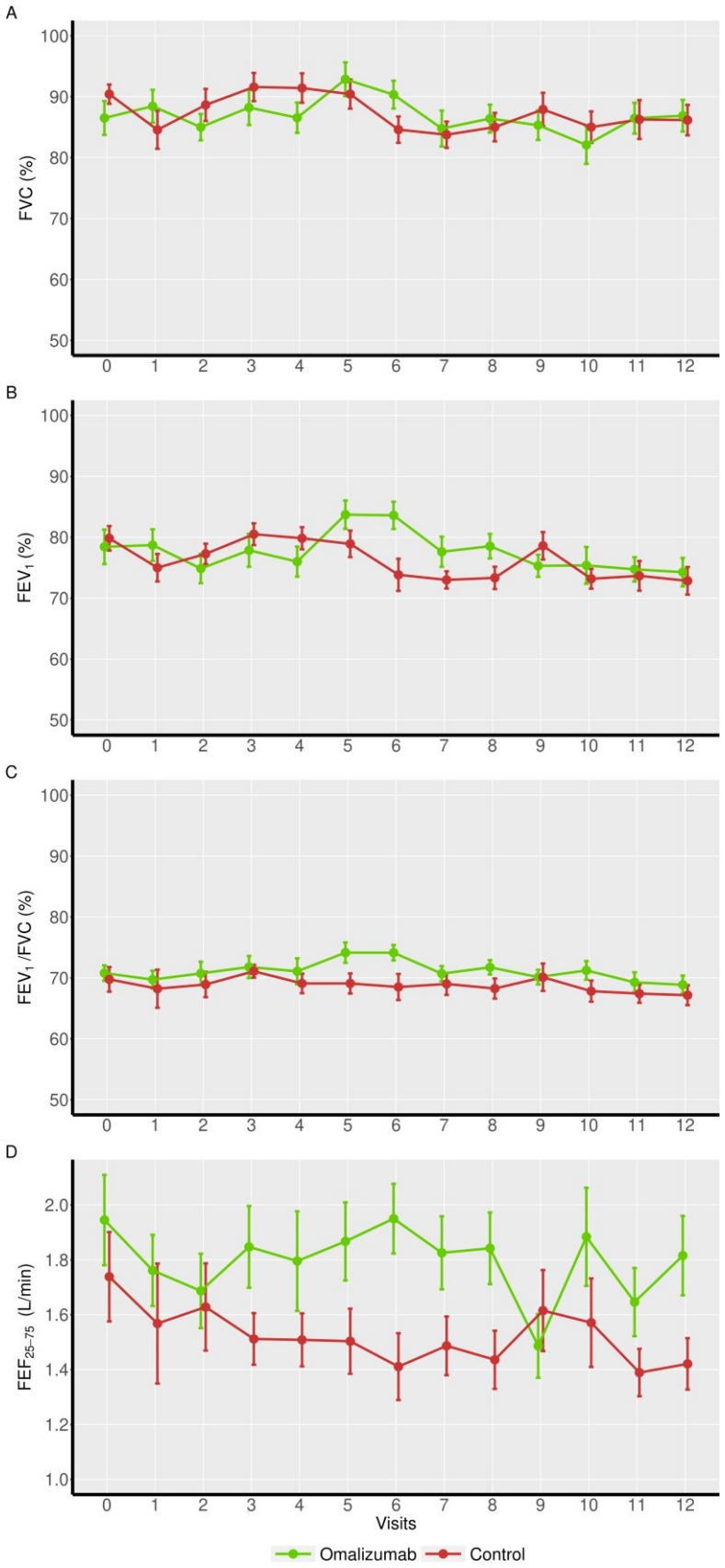
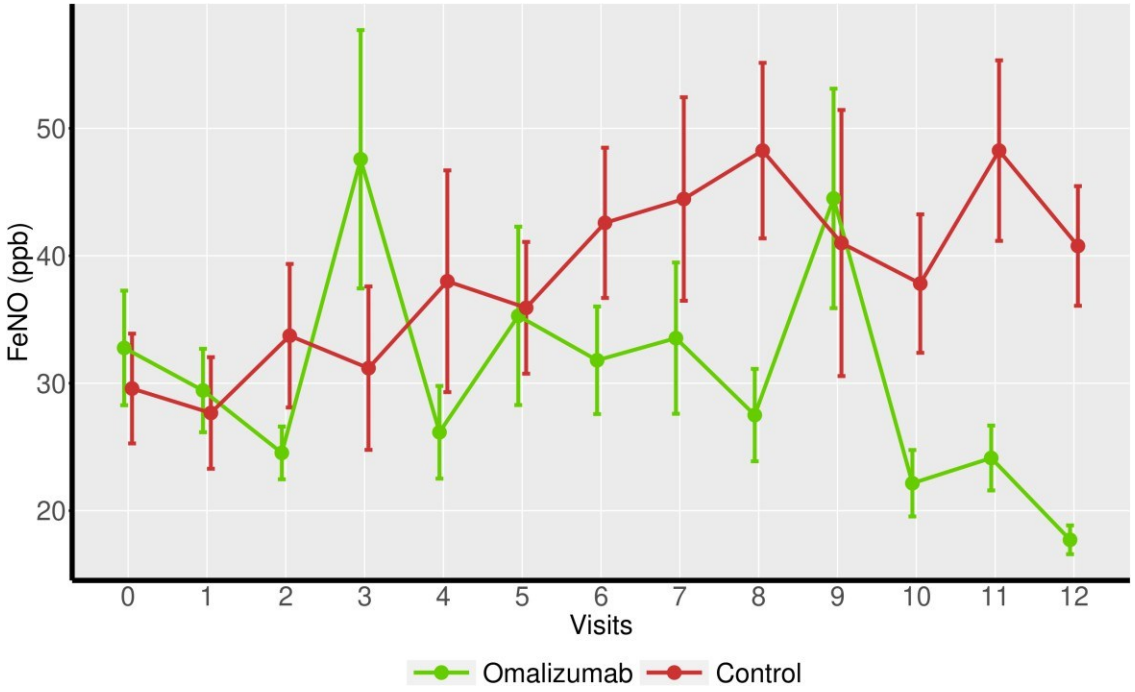


Figure 3s. Evolution of the FeNO values



This figure shows the evolution of the FeNO values throughout the study.

In the omalizumab group, there is a significant decrease in the inflammation. The FeNO value reaches normality at the end of the follow-up

Figure 4s. Total exacerbations during the study

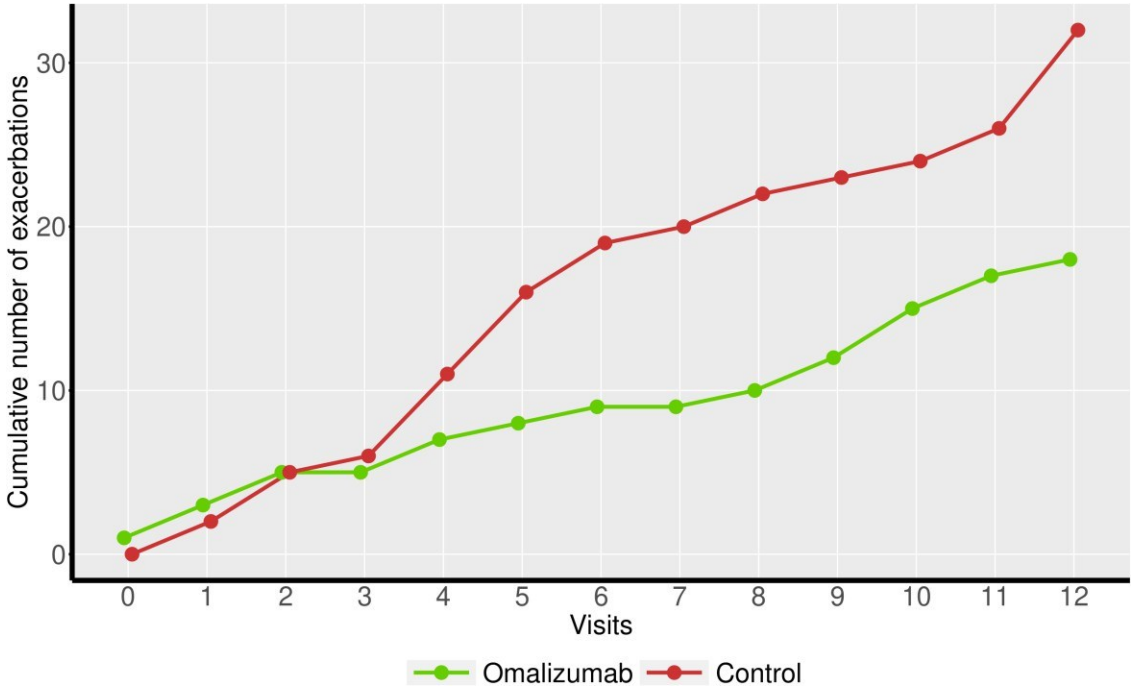


Figure 5s. Exacerbations by severity during the study

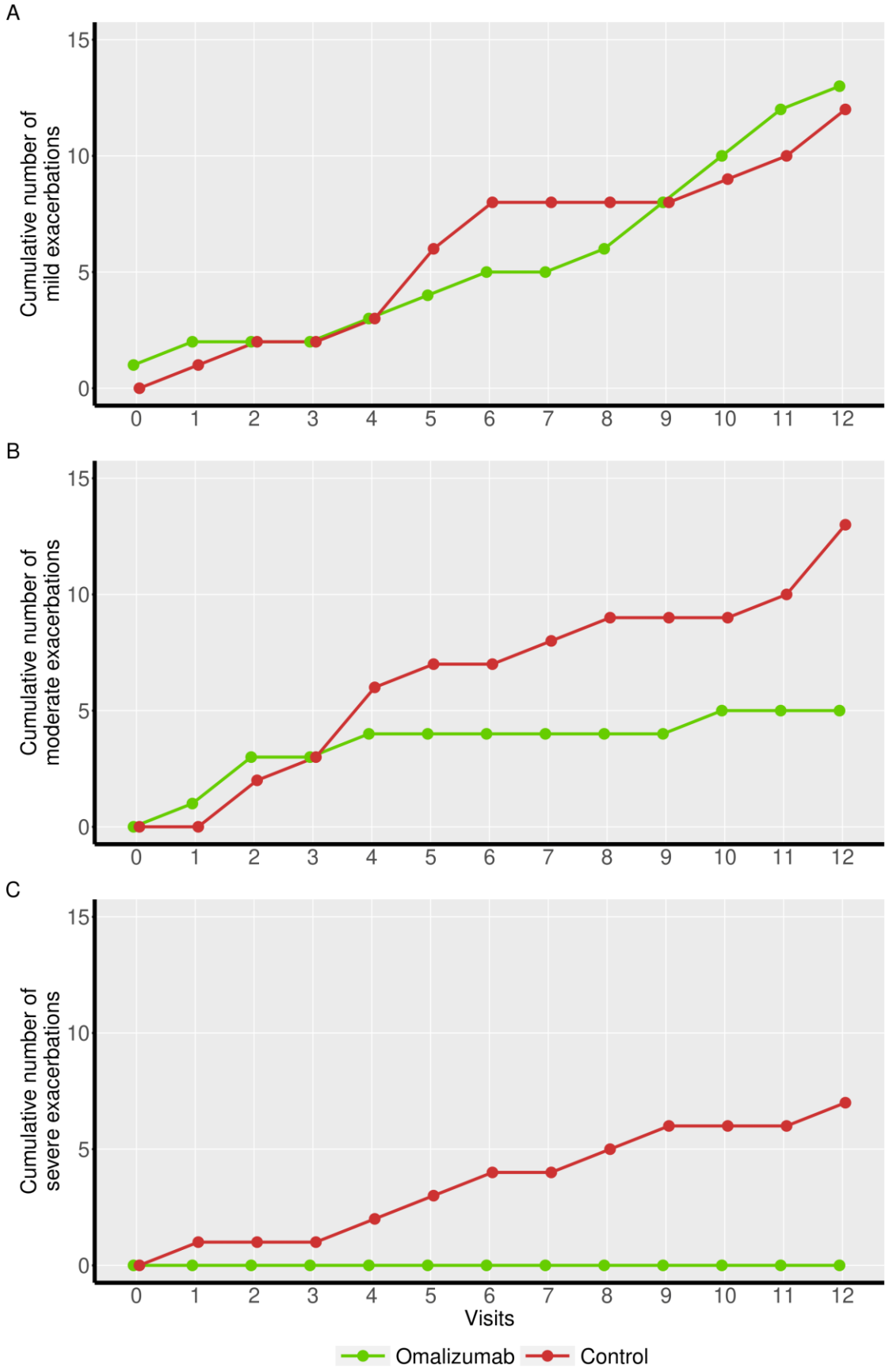


Figure 6s. Blood IgE evolution

Mean baseline IgE values were elevated in both groups, as all patients were allergic. In the omalizumab group, a 3.8-fold increase in blood IgE concentration was observed compared to the baseline level. The control group showed no significant changes

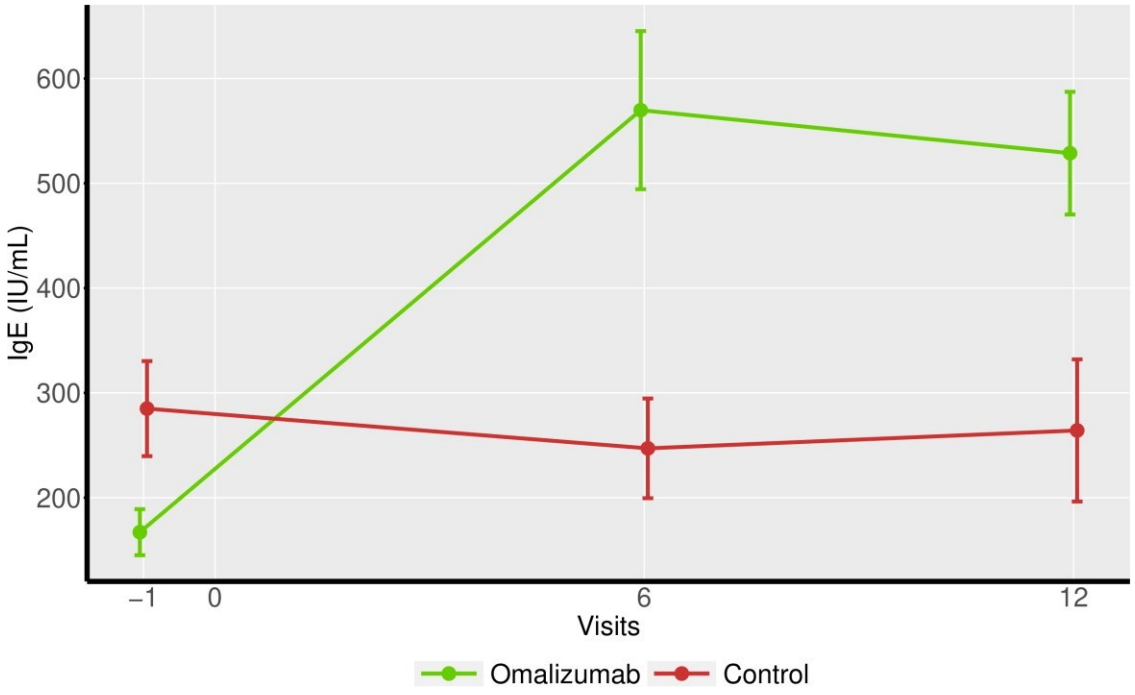


Figure 7s. Epithelium characteristics.

Scale of severity of the epithelium in biopsy 1 (beginning of treatment) and biopsy 2 (end of treatment). The damage ranges from mild (no detachment of the epithelium but increment of the inter-cellular space) to extreme severity (total loss of columnar epithelium).

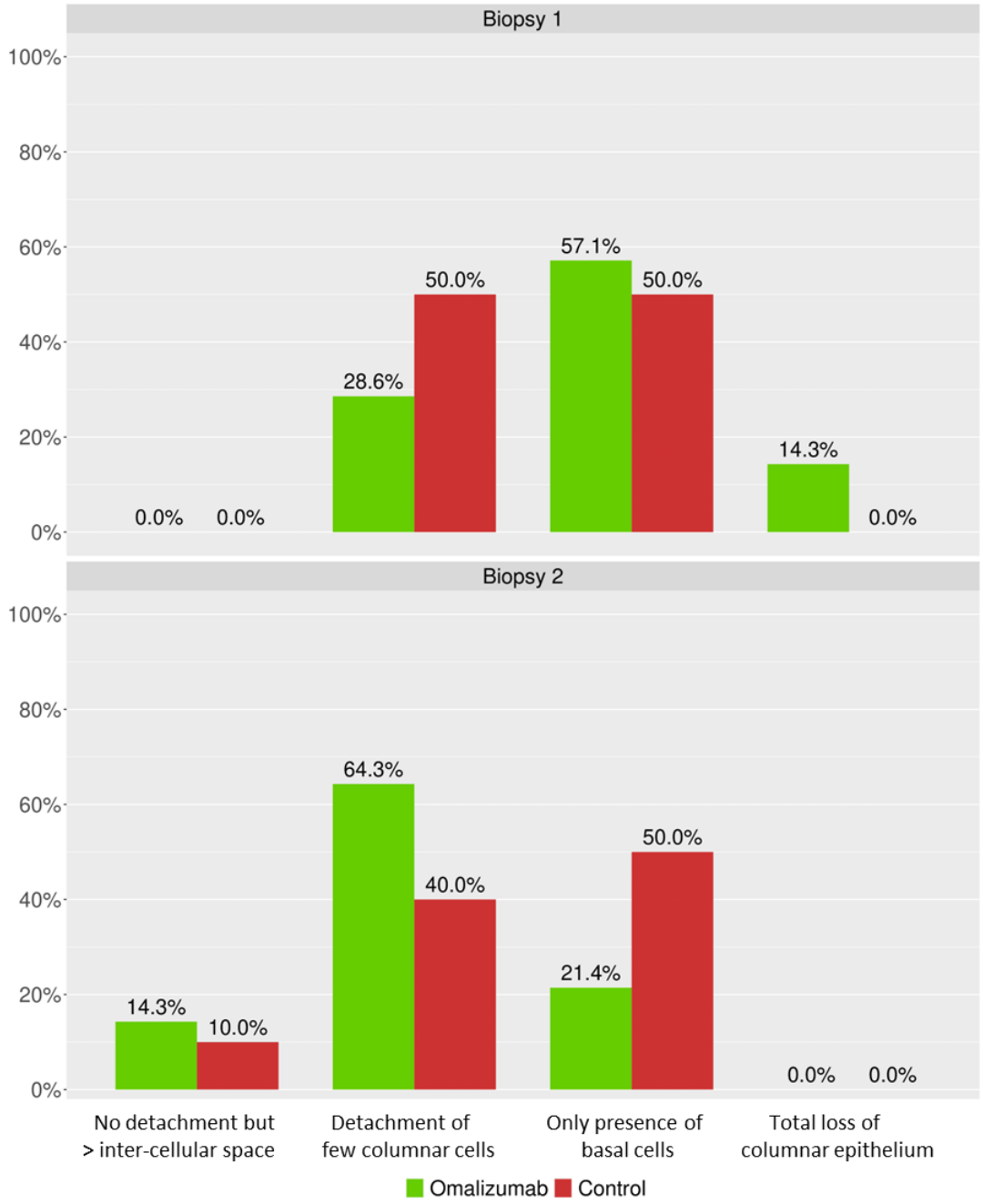


Figure 8s. Epithelium characteristics: improvement/no improvement

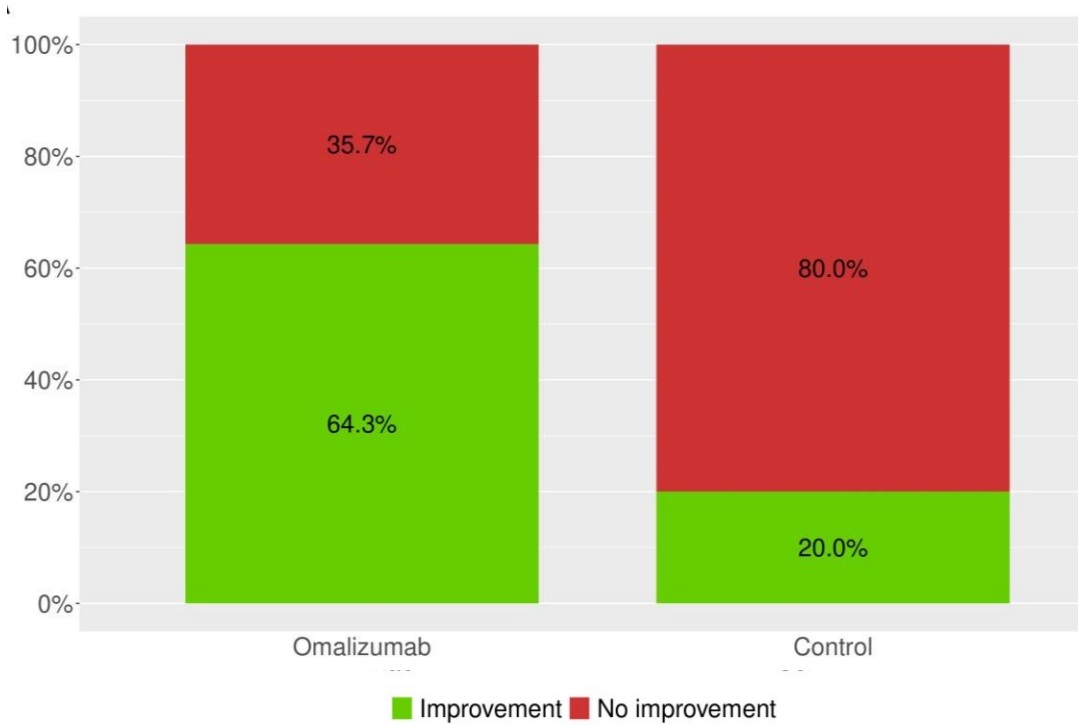


Figure 9s. Epithelium characteristics: focal and extensive damage

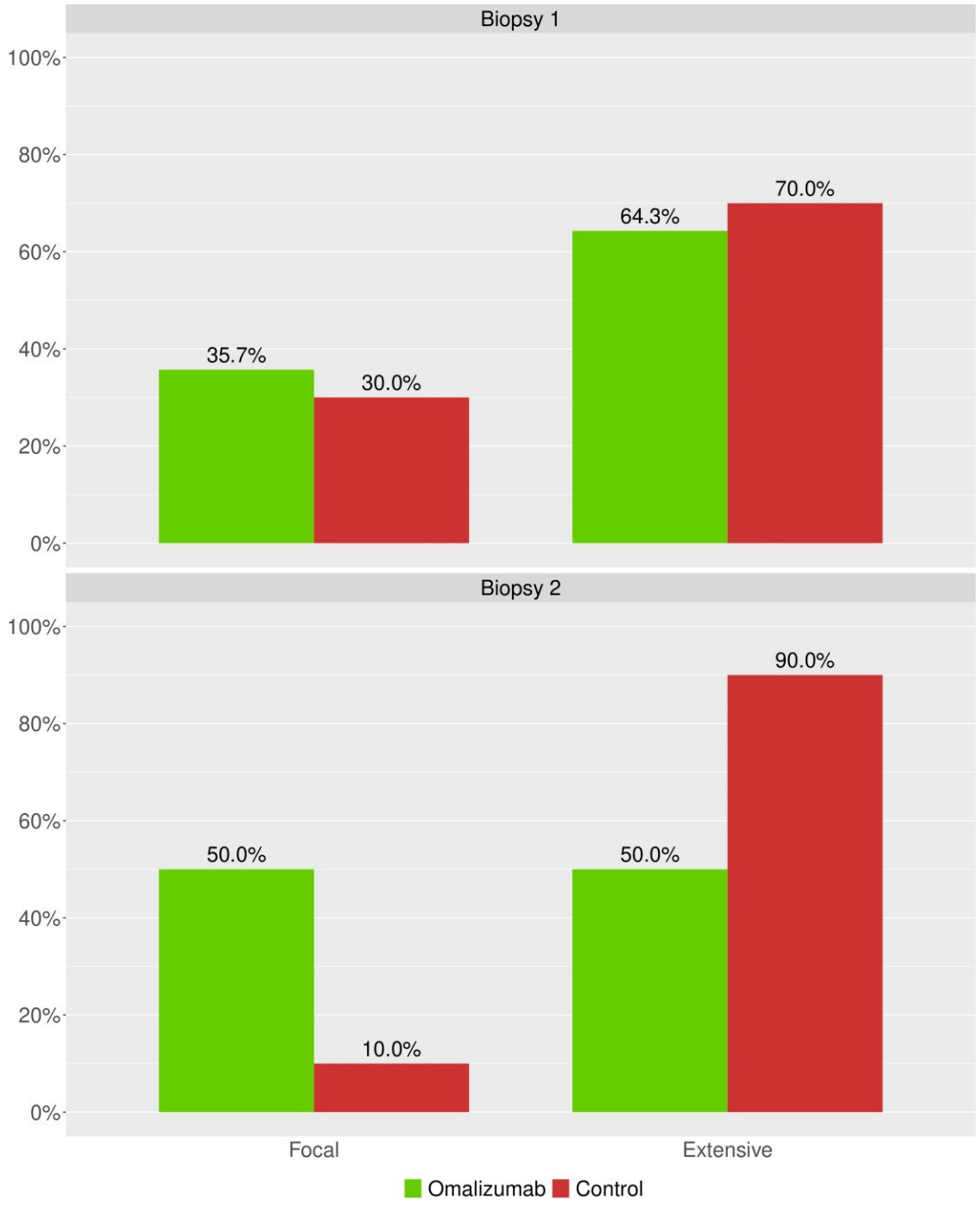
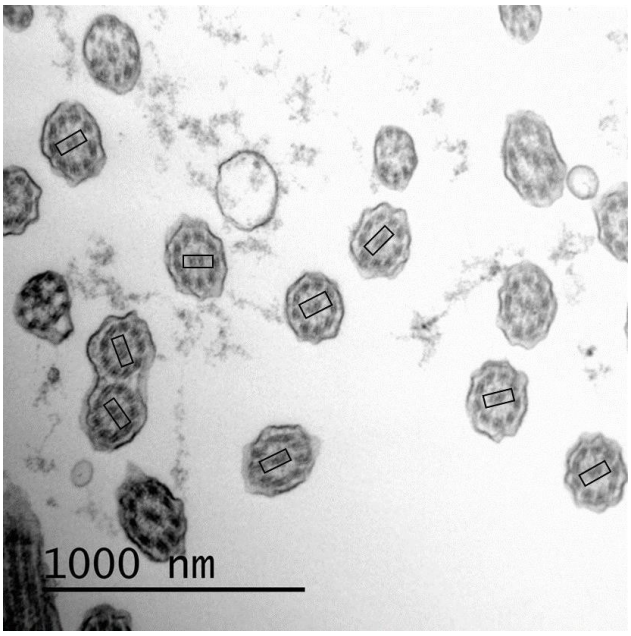


Figure 10s. Ciliary abnormalities. Cross-sectional image of cilia in patients treatment with omalizumab. Magnification, 20000x

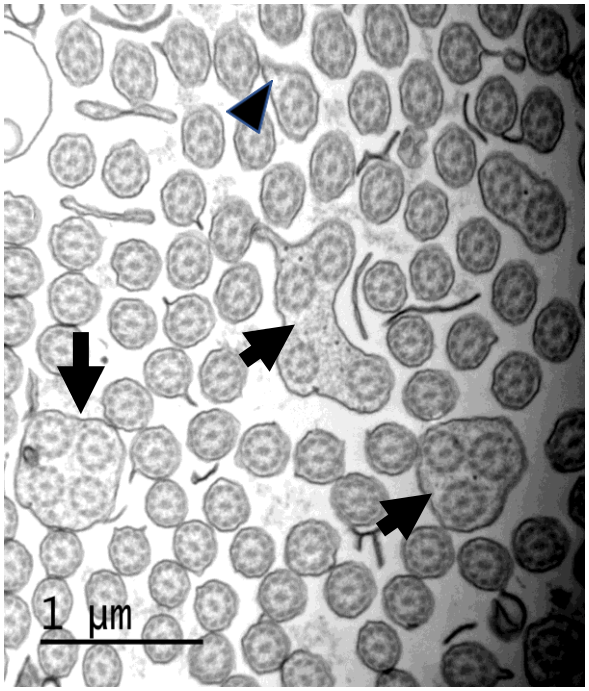
a- Observe the ciliary disorientation. The rectangles englobe the central pair of microtubules of the cilia. The angles of these axes vary more than 20º. Disorientation is considered when the orientation of the central axis of cilia differs more than 20º.

a



b- Observe ciliary abnormalities. Arrowheads show ciliary edema with increased periaxonemal matrix. Arrows show supernumerary microtubules.

b



11.- Adverse events.

No relevant drug related adverse events were detected.

None patient needed to abandon the study due to adverse events.

Adverse event	Present in the technical file of the medicinal product	Severe AE	Severity	Drug related	Treatment modification	Duration (days)	Active at end of study
Respiratory infection	NO	NO	Moderate	NO	NO	7	NO
Respiratory infection	NO	NO	Mild	NO	NO	9	NO
Seronegative arthritis	YES	NO	Moderate	NO	NO		YES
Steroid myopathy	NO	NO	Mild	NO	NO	54	NO
Restless legs	NO	NO	Mild	NO	NO	71	NO
Odynophagia	NO*	NO	Mild	NO	NO	35	NO
Arthromyalgia	YES	NO	Moderate	Probably	NO		YES
Dysphonia	NO*	NO	Mild	NO	NO	73	NO

(in bold patients on omalizumab).

*A similar side effect has been observed but named as pharyngitis rather than odynophagia or dysphonia, this is why we reported this side effect as not present in the technical file of the medicinal product.

Research in context

1. What is already known about this topic?

- The oral corticosteroid-sparing capacity of omalizumab is not adequately documented.
- The damage caused by severe asthma to bronchial histology (remodelling) is not well documented, nor is the ability of monoclonal antibodies to restore it.

2.- What does this article add to our knowledge.

- It is the first study to demonstrate the oral corticosteroid-sparing capacity of omalizumab.
- It describes the damage observed in the bronchial ciliated epithelium in patients with corticosteroid-dependent asthma studied by electron microscopy of bronchial biopsies
- It shows the restorative capacity of biological treatment on bronchial epithelium
- Contrary to previously held beliefs, it shows that bronchial remodelling in patients with severe asthma is reversible with biologic therapy despite a reduction in the dose of oral corticosteroids.
- It allows reinterpretation of the histological changes found, so that the thickening of the basement membrane appears to be a protective reaction of the epithelium to exert the barrier function lost with the damage of the disease.
- It reinforces the current idea of the importance of the bronchial epithelium in the pathophysiology of asthma (asthma as "epithelial driven disease").

3. How does this study impact current management guidelines

- It positions omalizumab in the group of corticoid-sparing monoclonal antibodies in severe allergic asthma**.**
- It supports the administration of biological treatments regardless of patient severity.

- It reinforces the use of monoclonal antibodies against oral corticosteroids

Corporació Parc Taulí

Doña Coloma Moreno Quiroga, Secretaria del Comité Ético de Investigación Clínica de la Corporació Sanitària Parc Taulí de Sabadell,

CERTIFICA:

Que en la sesión del día 10 de Noviembre de 2009, este Comité, actuando en calidad de CEIC de Referencia, ha evaluado la propuesta del promotor:

Efectividad clínica y cambios histológicos secundarios al tratamiento con omalizumab en asmáticos alérgicos en estadío V de la gina
Código: CHD-PI08
EudraCT: 2009-010914-31
Promotor: Dr. Christian Domingo

Versión de protocolo 19/10/2009 que incluye la enmienda 1
Hoja de información al paciente y consentimiento informado, de 19/10/2009
Hoja de Información al paciente y consentimiento informado para la cesión de muestras, de 19/10/2009

y considera que:

- El ensayo se plantea siguiendo los requisitos del Real Decreto 223/2004, de 6 de febrero y las normas que lo desarrollan y su realización es pertinente.
- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto, teniendo en cuenta los beneficios esperados.
- El seguro o la garantía financiera previstos son adecuados.
- El procedimiento para obtener el consentimiento informado, y el plan de reclutamiento de sujetos previstos son adecuados, así como las compensaciones previstas para los sujetos por daños que pudieran derivarse de su participación en el ensayo.
- La capacidad del investigador y sus colaboradores y las instalaciones y medios disponibles son apropiados para llevar a cabo el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.

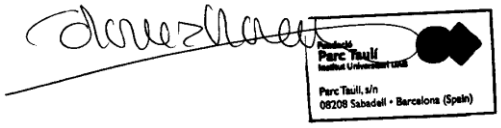
Este Comité, habiendo tenido en cuenta los informes recibidos de los CEIC implicados,

Emite un DICTAMEN FAVORABLE para la realización de dicho ensayo en España para los siguientes Centros:

- Corporació Sanitària Parc Taulí. Dr. Christian Domingo
- Hospital de Sant Boi. Dr. Luis Lores
- Hospital de Pontevedra. Dr. Francisco Javier González Barcala
- Hospital Germans Trias i Pujol. Dr. Carlos Martínez Rivera

El Hospital Mútua de Terrassa emite informe desfavorable a la participación en el estudio aspectos locales por desacuerdo en los procedimientos que se realizan y la no aportación de la medicación por parte del promotor.

Este Comité tiene conocimiento de que el estudio se realizará en la Clínica Meritxell por el Dr. Jordi Roig



Sabadell, 10 de Novembre de 2009
2009048

HOJA DE INFORMACIÓN AL PACIENTE

Título del estudio: Eficacia clínica y cambios histológicos secundarios al tratamiento con omalizumab en asmáticos alérgicos en estadio V de la GINA.
Promotor del estudio: Dr. Christian Domingo
Código del estudio: CHD-PI08

Investigador Principal: _____

Por favor, lea atentamente esta hoja de información:

En estos momentos se le está proponiendo su participación en un estudio de investigación español, que ayudará a aumentar el conocimiento sobre la eficacia clínica y los cambios histológicos secundarios al tratamiento con omalizumab en asmáticos alérgicos en estadio V de la GINA [2] ***(Iniciativa Global para el Asma).***

Propósito y realización del estudio

[1]***El propósito del estudio es valorar en los pacientes asmáticos dependientes de corticoides la efectividad clínica de Xolair® en la disminución de la dosis de corticoides orales sin deterioro de la función respiratoria, los cambios en la inflamación de la vía aérea, los cambios en la mucosa bronquial, la tolerância al fármaco y los posibles efectos secundarios asociados que puedan aparecer.***

Su participación consistirá en el registro de datos relacionados con su asma.

Usted continuará tomando Urbasón de acuerdo a los criterios clínicos habituales de acuerdo con la opinión de su médico y se le asignará o no un tratamiento adicional ya comercializado para el asma (Xolair®). [4]**La asignación a los grupos de tratamiento será aleatoria.**

Urbasón® es un corticoesterioide de acción antiinflamatoria e inmunosupresora que está indicado para el tratamiento del asma persistente grave.

Xolair® es un anticuerpo monoclonal de acción reductora de la respuesta alérgica que está indicado para el tratamiento del asma persistente grave.

Cuando vuelva al hospital, el médico del estudio le evaluará la evolución de su asma, si ha mejorado o empeorado, y si ha tenido algún efecto secundario relacionado con el mismo.

Su participación en el estudio durará un año. Durante este tiempo, el médico le solicitará que acuda al hospital, además del día que acepte participar en el estudio, una vez al mes.[3]

Como parte del control de su asma, durante el estudio se le practicarán evaluaciones de rutina como extracción de sangre, [2]***prick test (prueba cutánea para valorar posibles agentes causantes de alergia)***, pruebas de función pulmonar y espirometría. Se le realizarán también dos broncoscopias con biopsia bronquial. [3]***Para la realización de la broncoscopia se requiere el uso de un dispositivo para observar el interior de los pulmones. A través del dispositivo se le extraerá una muestra pequeña de tejido (biopsia) de los pulmones.*** Habitualmente la broncoscopia no da lugar a complicaciones[3]. Las incidéncias más frecuentes de la broncoscopia son el neumotórax (salida de aire del pulmón hasta la pleura) en cuyo caso requeriría su

[1.] *Cambios solicitados por el CEIC Hospital Parc Taulí (CEIC de referéncia)*
[2.] *Cambios solicitados por el CEIC IMAS*
[3.] *Cambios solicitados por el CEIC SERGAS*
[4.] *Cambios solicitados por el CEIC Hospital Mútua de Terrassa*

hospitalización y la hemoptisis (expulsión de sangre con la tos). [2] ***Las broncoscopias y biopsias que se le realizarán no forman parte de la práctica clínica habitual.***

Además, durante ese tiempo, es posible que él/ella o algún miembro de su equipo le llamen por teléfono para preguntarle por su evolución.

[1] En este estudio está previsto incluir un total de 58 pacientes. Las guías internacionales para el tratamiento del asma persistente grave indican como tratamientos, independientemente de su participación en el estudio: Urbasón® o Xolair®. La participación en este estudio no supone ningún cambio respecto al seguimiento o tratamiento de su enfermedad ya que en caso que no acepte participar en el estudio, el tratamiento que se le prescribirá será Urbasón® y/o Xolair®.

Participación voluntaria

Su participación es totalmente voluntaria. Es usted libre de rechazar colaborar en este estudio, sin que tenga que explicar los motivos que le llevan a tomar esa decisión y sin que se vea afectada su atención médica. [3]***Puede consultar con otras personas antes de tomar una decisión sobre su participación en el estudio.*** [2]***Usted puede retirarse del estudio en cualquier momento y sin dar explicaciones.***

Su nombre e iniciales no aparecerán en ningún documento del estudio. Usted será identificado exclusivamente por un número, para garantizar la confidencialidad de sus datos. El tratamiento, la comunicación y la cesión de los datos de carácter personal se ajustarán a lo dispuesto en la Ley Orgánica de protección de datos de carácter personal 15/1999 de 13 de diciembre. De acuerdo con esta ley, usted puede ejercer el derecho de acceso, modificación, oposición y cancelación de datos, para lo cual deberá dirigirse a su médico.

Póliza de seguro

El Promotor del estudio ha concertado una póliza de seguros (número 130/001/006226) con la compañía HDI Hannover Internacional (España) Seguros y Reaseguros S.A. Que se ajusta a la legislación vigente y que cubre todos los posibles daños y lesiones que pudieran producirse en relación con su participación en el estudio.

[1] Riesgos de participación

La participación en este estudio puede generar reacciones adversas asociadas a Urbasón® o Xolair®.

Las reacciones adversas más frecuentes asociadas al uso a largo plazo de Urbasón® son cambios reversibles en la distribución de las grasas corporales, aumento en los niveles de azucar en sangre, acumulación de agua en los tejidos, cambios en la piel, enlentecimiento de la cicatrización de las heridas u osteoporosis por esteroides.

Las reacciones adversas más frecuentes asociadas a Xolair® son cefalea, reacciones en el lugar de la inyección (dolor, eritema, prurito, tumefacción). Menos frecuentes son incremento de peso, fatiga, nauseas, diarrea o tos. En casos muy aislados se ha descrito anafilaxia [4] que es una reacción alérgica severa en todo el cuerpo.

En caso de riesgos no descritos hasta ahora que durante el transcurso de la investigación se

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2. *Cambios solicitados por el CEIC IMAS*
3. *Cambios solicitados por el CEIC SERGAS*
4. *Cambios solicitados por el CEIC Hospital Mútua de Terrassa*

obtengan nuevos datos relacionados con el tratamiento que ha elegido, el médico del estudio se los comunicará. Sería el caso de reacciones no previsibles y desconocidas hasta la fecha.

Beneficios de participar

El hecho de proporcionar sus datos médicos para este estudio puede no beneficiarle directamente, pero la información obtenida puede ser utilizada para apoyar la evidencia científica de la eficacia y seguridad del tratamiento con Xolair® en la practica clínica habitual.

Compensación

No se percibirá ninguna compensación económica por la participación en este estudio.

Confidencialidad de los datos

El acceso a su información personal quedará restringido a su médico y colaboradores, y si lo requieren para comprobar los datos y procedimientos del estudio, al Comité Ético de Investigación Clínica que ha aprobado el estudio y al promotor del mismo, pero siempre manteniendo la confidencialidad de sus datos según la legislación vigente.

[1]Si acepta participar en el estudio, los datos obtenidos a lo largo del mismo serán tratados de forma confidencial en todo momento y por cualquier persona autorizada a su acceso. Los datos originales serán conservados en el centro del investigador y sólo tendrán acceso los investigadores del estudio, la/s persona/s encargada/s de su monitorización, el Comité Ético de Investigación Clínica y las Autoridades Sanitarias Españolas.

Usted será identificado mediante un código de paciente y los datos obtenidos en el presente estudio seguirán las regulaciones españolas sobre el manejo de datos computerizados (L.O. 15/1999, de 13 de Diciembre de Protección de Datos de Carácter Personal).

Los resultados de este estudio se presentarán en publicaciones o comunicaciones en congresos.
En ningún caso se le identificará en estas publicaciones.
Debe saber que este estudio ha sido aprobado por un Comité Ético de Investigación Clínica, y se realizará cumpliendo la legislación vigente en España para este tipo de estudios.

Si tiene alguna duda relativa al estudio, por favor consulte a su médico.

Nombre del médico: ……

Teléfono de contacto: ……

[1] Se entregará una copia de la hoja de información al paciente y consentimiento informado al paciente.

[1.] *Cambios solicitados por el CEIC Hospital Parc Taulí (CEIC de referéncia)*
[2.] *Cambios solicitados por el CEIC IMAS*
[3.] *Cambios solicitados por el CEIC SERGAS*
[4]. *Cambios solicitados por el CEIC Hospital Mútua de Terrassa*

FORMULARIO DE CONSENTIMIENTO INFORMADO

Título del estudio: Eficacia clínica y cambios histológicos secundarios al tratamiento con omalizumab en asmáticos alérgicos en estadio V de la GINA.
Promotor del estudio: Dr. Christian Domingo
Código del estudio: CHD-PI08

Yo, (nombre y apellidos) .....

He leído la hoja de información sobre la efectividad clínica y cambios histológicos secundarios al tratamiento con omalizumab en asmáticos alérgicos en estadio V de la GINA.

- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.
- He hablado con el doctor .....

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:

- Cuando quiera
- Sin tener que dar explicaciones
- Sin que esto repercuta en mis cuidados médicos.

Entiendo que al acceder a participar en este estudio, consiento en la recogida, tratamiento, cesión y transferencia (si procede) de mis datos personales, con respeto al anonimato, para fines de atención sanitaria y/o investigación médica

Presto libremente mi conformidad para participar en el estudio y que mis datos puedan ser utilizados con fines de investigación.

Firma del/la participante: ..... Fecha: _____/_____/_____
Día *Mes* *Año*

Firma del investigador: ..... Fecha: _____/_____/_____
Día *Mes* *Año*

Por favor, recuerde que el formulario ha de ser completado POR EL/LA PARTICIPANTE, de su puño y letra [1] ***El paciente debe recibir una copia de la hoja de información al paciente y consentimiento informado***
Muchas gracias por su colaboración

[1.] *Cambios solicitados por el CEIC Hospital Parc Taulí (CEIC de referéncia)*
[2.] *Cambios solicitados por el CEIC IMAS*
[3.] *Cambios solicitados por el CEIC SERGAS*
[4]. *Cambios solicitados por el CEIC Hospital Mútua de Terrassa*

HOJA DE INFORMACIÓN AL PACIENTE PARA CESIÓN DE MUESTRAS

Título del estudio: Eficacia clínica y cambios histológicos secundarios al tratamiento con omalizumab en asmáticos alérgicos en estadio V de la GINA.

Promotor del estudio: Dr. Christian Domingo

Código del estudio: CHD-PI08

Investigador Principal: _____

Por favor, lea atentamente esta hoja de información:

El PROMOTOR del estudio pretende desarrollar un proyecto de investigación denominado CHD-PI08 que tiene como objetivo principal profundizar en el conocimiento de la efectividad clínica y cambios histológicos secundarios al tratamiento con omalizumab en pacientes con asma grave persistente. Este hospital es uno de los centros participantes en este proyecto de investigación.

Este estudio requiere la utilización de muestras biológicas obtenidas de sujetos que reúnen una serie de condiciones previas definidas en el protocolo por el promotor. Para ello, se le van a extraer dos biopsias bronquiales que serán enviadas a la Universidad Autónoma de Barcelona (Bellaterra) y al laboratorio de anatomía patológica del Hospital Quirón de Barcelona para ser analizadas. Este tejido es un valioso instrumento con destino a la investigación del asma grave persistente, que puede permitir la obtención de conocimientos que sirvan para la obtención y desarrollo de nuevas estrategias y terapias aplicables a pacientes.

Considerando la patología que padece y las condiciones que reúne, Vd. es un sujeto idóneo para participar en este proyecto de investigación.

Por ello, solicitamos su consentimiento para la extracción de dos biopsias bronquiales a través de broncoscopia que están destinadas a este proyecto de investigación.

RIESGOS Y MOLESTIAS

Las molestias y riesgos de participar en este estudio están relacionados con la obtención de las biopsias mediante broncoscopia.

RIESGOS Y MOLESTIAS DEL PROCEDIMIENTO PARA LA OBTENCIÓN DE LAS MUESTRAS:

Habitualmente la broncoscopia no da lugar a complicaciones[4]. Las incidencias más frecuentes de la broncoscopia son el neumotórax (salida de aire del pulmón hasta la pleura) en cuyo caso requeriría su hospitalización y la hemoptisis (expulsión de sangre con la tos). Las broncoscopias y biopsias que se le realizarán no forman parte de la práctica clínica habitual de su condición.

[4]. Cambios solicitados por el *CEIC del Hospital Mutua de Terrassa*

BENEFICIO Y ATENCIÓN MÉDICA

La cesión de muestras para investigación es voluntaria y altruista. Su único beneficio es el que corresponde al avance de la medicina en beneficio de la sociedad, y el saber que ha colaborado en este proceso.
La muestra así recogida no podrá ser objeto directo de actividades con ánimo de lucro. No obstante, la información generada a partir de los estudios realizados sobre su muestra podría ser fuente de beneficios comerciales. En tal caso, están previstos mecanismos para que estos beneficios reviertan en la salud de la población, aunque no de forma individual en el donante. Su participación en este estudio es completamente voluntaria. Si usted decide no participar recibirá todos los cuidados médicos que pudiera necesitar y su relación con los equipos médicos que le atiendan no se verá afectada.

DESTINO DE LA MUESTRA Y CESIÓN A OTRAS LINEAS DE INVESTIGACIÓN

Las muestras obtenidas no serán cedidas a terceros ni serán utilizadas con fines distintos a este proyecto de investigación.
El análisis morfológico de las biopsias bronquiales se realizará en el laboratorio para Microscopía electrónica en la Universidad Autónoma de Barcelona (Bellaterra, Barcelona).
El análisis inmunohistoquímico se realizará en el laboratorio de anatomía patológica del Hospital Quirón de Barcelona
El análisis inmunohistoquímico será información sobre el Colágeno IV y el Factor de Crecimiento Epidérmico.

Las muestras sobrantes serán destruidas una vez hayan sido analizadas.

Su médico, junto con la muestra, recogerá aquellos datos personales, como edad, sexo, raza, así como los datos de salud directamente relacionados con la patología por la que cual se le recoge la muestra para investigación. Sólo el MEDICO RESPONSABLE DE SUS CUIDADOS MÉDICOS podrá relacionar estos datos con Vd.
El hospital registrará los datos que puedan identificarle con las muestras antes de ser enviadas para ser analizadas, empleando un procedimiento de encriptación y codificación.
Asimismo, sólo su médico será el responsable de custodiar el documento de consentimiento, y sólo a él le corresponde garantizar el cumplimiento de su voluntad en relación al uso de la muestra biológica que Vd. cede a para investigación.

Su médico garantiza que en ningún caso saldrá del centro dato alguno que le identifique personalmente.

[4]. Cambios solicitados por el *CEIC del Hospital Mutua de Terrassa*

REVOCACIÓN DEL CONSENTIMIENTO

En cualquier momento podrá Vd. revocar el consentimiento para utilizar las muestras obtenidas, pudiendo solicitar la destrucción o la anonimización de las mismas. No obstante, los efectos de la revocación no se extenderán a los datos resultantes de las investigaciones que se hayan llevado a cabo previamente a la misma.

Puede ejercitar su derecho de acceso, rectificación y cancelación de dicha información dirigiéndose al Dr. _____, responsable de la realización del estudio en el centro.

En el caso de anonimización, se romperá irreversiblemente todo vínculo que permita relacionar las muestras y los datos almacenados con su persona.

DERECHOS Y GARANTÍAS

Usted tiene derecho a conocer los datos que se obtengan a partir del análisis de las muestras de biopsia donadas.

Como consecuencia de la investigación puede obtenerse información relativa a su salud derivada de los análisis que se realicen sobre su muestra biológica. Vd. puede decidir si desea recibir esta información, o si por el contrario renuncia a este derecho.

Asimismo, se le indica que la información que se obtenga puede implicar a sus familiares, siendo únicamente Vd. el responsable de facilitar, en su caso, información a los mismos, o proporcionar a las personas responsables del estudio los datos precisos para su localización y contacto.

CONFIDENCIALIDAD

Con la firma de este consentimiento, Vd. autoriza al investigador y al promotor, junto con el empleo de la muestra *biológica*, a recoger y procesar los datos personales agregados a la muestra, como son: fecha de nacimiento, sexo, origen étnico, y datos sobre su condición, relevantes para los fines de esta investigación. Estos datos se someterán a un proceso de disociación mediante la asignación de un código, para garantizar la protección de su identidad.

Su médico y el promotor, en su caso, utilizarán estos datos para la realización de esta investigación. Igualmente, el médico y el promotor podrán utilizar sus datos para apoyar ante las autoridades competentes o en las presentaciones públicas que realicen, los resultados de la investigación.

Los resultados del estudio podrán ser comunicados en reuniones científicas, congresos médicos o publicaciones científicas. Siempre se mantendrá una estricta confidencialidad sobre su identidad.

Tanto el médico como el Promotor son responsables del manejo de los Datos del Estudio, conforme el Real Decreto 223/2004 y la Ley Orgánica 15/1999, de 13 de diciembre, sobre Protección de Datos de Carácter Personal y el Real Decreto 1720/2007, de 21 de diciembre, por el que se aprueba el Reglamento de desarrollo de la Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal.

Asimismo se dará cumplimiento a los requerimientos de la Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y

4. Cambios solicitados por el *CEIC del Hospital Mutua de Terrassa*

documentación clínica, y la Ley 14/2007, de 3 de julio, de Investigación biomédica.

Los derechos de acceso, rectificación, cancelación y oposición puede ejercitarlos ante el Dr. ……..

Si tiene alguna duda relativa al estudio, por favor consulte a su médico.

Nombre del médico: ……

Teléfono de contacto: ……

Se entregará una copia de la hoja de información al paciente y consentimiento informado al paciente.

[4]. Cambios solicitados por el *CEIC del Hospital Mutua de Terrassa*

CONSENTIMIENTO INFORMADO PARA LA CESIÓN DE MUESTRAS

Título del estudio: Eficacia clínica y cambios histológicos secundarios al tratamiento con omalizumab en asmáticos alérgicos en estadio V de la GINA.

Promotor del estudio: Dr. Christian Domingo

Código del estudio: CHD-PI08

Investigador Principal:.....

Yo (paciente) ……..…..…..…

con DNI…..……..…… declaro bajo mi responsabilidad que he leído la hoja de Información al paciente y acepto participar en este estudio.

- Se me ha entregado una copia del documento "Hoja de Información al paciente para la cesión de muestras". En ella se me han explicado las características y el objetivo del estudio, así como los posibles beneficios y riesgos que puedo esperar.
- Se me ha dado tiempo y oportunidad para realizar preguntas. Todas las preguntas respondidas a mi entera satisfacción.
- Sé que se mantendrá en secreto mi identidad y que se identificarán mis muestras con un sistema de codificación.
- Soy libre de retirarme del estudio en cualquier momento por cualquier motivo, sin tener que dar explicación y sin que repercuta negativamente sobre cualquier tratamiento médico presente o futuro.
- Tras ello se procederá a la destrucción de la muestra codificada.
- Entiendo la finalidad del estudio y que los resultados del mismo no se comunicarán, excepto en el caso de que dichos hallazgos tengan una implicación significativa para la salud de los participantes y que exista una posibilidad real de mejorar esa condición de salud.

Yo **DOY mi consentimiento** para que se utilicen mis muestras y los datos asociados como parte de este proyecto de investigación. Consiento en participar voluntariamente y renuncio a reclamar cualquier beneficio económico por mi participación en el estudio.

Por la presente afirmo haber sido advertido sobre la posibilidad de recibir información relativa a mi salud derivada de los análisis que se realicen sobre mi muestra biológica.

□ **Yo solicito información**

□ **Yo no quiero recibir información**

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una vez finalizada la investigación sobre los resultados del estudio.

Firma del/la participante: ..... Fecha: _____/_____/_____
Día Mes Año

Constato que he explicado las características del proyecto de investigación y las condiciones de conservación y seguridad que se aplicarán a la muestra y a los datos conservados.
Constato que he explicado las características de la cesión de muestras y la posible utilización de las mismas, así como las condiciones de conservación y seguridad que se aplicarán a la muestra y a los datos conservados.

Firma del investigador: ..... Fecha: _____/_____/_____
Día Mes Año

Por favor, recuerde que el formulario ha de ser completado POR EL/LA PARTICIPANTE, de su puño y letra ***El paciente debe recibir una copia de la hoja de información al paciente y consentimiento informado***
Muchas gracias por su colaboración

4. Cambios solicitados por el *CEIC del Hospital Mutua de Terrassa*

EXPERIOR	NOTIFICACIÓN DE ACONTECIMIENTOS ADVERSOS GRAVES	Página 1 de 5

Código de Protocolo		Número Centro		Número Paciente	
Número de referencia local (A cumplimentar por Experior)					

SECCIÓN 1.- TIPO DE INFORME.	Inicial ☐	Seguimiento ☐

SECCIÓN 2.- DATOS DEMOGRÁFICOS DEL PACIENTE.

Código de Protocolo	Número de centro	Número de paciente	Sexo	Fecha de nacimiento (dd/mmm/aaaa)	Estatura (cm)	Peso (Kg.)

SECCIÓN 3.- ACONTECIMIENTO ADVERSO GRAVE.

SECCIÓN 3.1.- DATOS DEL ACONTECIMIENTO ADVERSO.

ACONTECIMIENTO ADVERSO	FECHAS (dd/mmm/aaaa)		RELACIÓN con el medicamento	DESENLACE del AAG	ACCIÓN llevada a cabo con el medicamento
Enumere todo signo o síntoma o el diagnostico final (si se conoce)	Inicio (dd/mmm/aaaa)	Fin (dd/mmm/aaaa)	1= No relacionado 2= Improbable 3= Posible 4= Probable 5= Definitiva	1= Resuelto 2= Secuelas 3= Mejora 4= Empeora 5= Muerte 6= Sin cambios	1= Interrumpida 2= Discontinuada 3= Disminución dosis 4= Aumento de dosis 5= Sin Cambios 6= No disponible

SECCIÓN 3.2.- GRAVEDAD.- Por favor, indique el (los) criterio(s) de gravedad del acontecimiento adverso, tantos como aplique.

☐ Muerte ☐ Requiere hospitalización ☐ Incapacidad permanente o significativa

☐ Amenaza de vida ☐ Prolongación hospitalización ☐ Anomalía o Malformación congénita

☐Otros/ Clínicamente relevante (por favor especifique): _____

SECCIÓN 3.3.- CAUSAS del Acontecimiento Adverso.- Escoja tantos como aplique.

☐ Condiciones médicas (por favor, rellene la sección 8) ☐ Medicación concomitante (por favor rellene la sección 7)

☐ Actividades relacionadas con la participación en el estudio (procedimientos del protocolo)

☐Otros (por favor especifique): _____

SECCIÓN 3.4.- RELACIÓN CON EL PRODUCTO EN INVESTIGACIÓN.- Explique la relación con el producto en investigación / el criterio del investigador. (a cumplimentar cuando la relación con el producto en investigación se haya calificado como "posible", "probable" o "definitiva")

En caso de que se haya suministrado más de un producto en investigación, por favor especifique, cuál de ellos podría haber contribuido al acontecimiento adverso.

POR FAVOR, ENVÍE <u>URGENTEMENTE</u> A FARMACOVIGILANCIA de EXPERIOR
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Código de Protocolo		Número Centro		Número Paciente	
Número de referencia local (A cumplimentar por Experior)					

SECCIÓN 3.5.- DESCRIPCIÓN DEL ACONTECIMIENTO ADVERSO.- Por favor, describa la posible reacción adversa de forma completa. Esta descripción puede acompañarse de cuántos informes considere necesarios para la adecuada interpretación del cuadro clínico sospechoso de ser una reacción adversa (sí lo necesita, use la sección 9)

SECCIÓN 4.- DATOS DEL PRODUCTO EN INVESTIGACIÓN.- (Si se utilizan más de dos productos en investigación, anote los datos en la sección 9 de información adicional.

Medicamento en Investigación	Sospechoso (Si/No)	Dosis (unidades)	Pauta	Vía de admin.	Fechas (dd/mmm/aaaa)		Indicación
					Inicio	Fin	

¿Remitió la reacción tras la interrupción del tratamiento o la reducción de la dosis del(los) fármaco(s) sospechoso(s)?

Sí ☐ **No** ☐ **No procede** ☐ **No disponible** ☐

¿Reapareció la reacción tras la reanudación del tratamiento del(los) fármaco(s) sospechoso(s)?

Sí ☐ **No** ☐ **No procede** ☐ **No disponible** ☐

"Sospechoso" es todo medicamento que podría haber contribuido a la aparición del (de los) acontecimiento(s) adverso (s) notificado(s).

SECCIÓN 5. -MEDICACIÓN CONCOMITANTE.-Anote la medicación concomitante que tomaba el paciente cuando se produjo el (los) acontecimiento(s) adverso(s).Ponga N/A si el paciente no estaba en ningún otro tratamiento, N/D si no conoce dicha medicación.

Medicamento en Investigación	Sospechoso (Si/No)	Dosis (unidades)	Pauta	Vía de admin.	Fechas (dd/mmm/aaaa)		Indicación
					Inicio	fin	

"Sospechoso" es todo medicamento concomitante (aparte del producto en investigación) que podría haber contribuido a la aparición del (de los) acontecimiento(s) adverso (s) notificado(s).

SECCIÓN 6.- DIAGNÓSTICO Y TRATAMIENTO DEL ACONTECIMIENTO ADVERSO

SECCIÓN 6.1.-DIAGNOSTICO.- ¿Se realizó alguna prueba diagnóstica (p.ej., radiografías, TAC, RMN, biopsias, cultivos, analíticas) de interés para el(los) acontecimiento(s) adverso(s)?

Sí ☐ **No** ☐ **No procede** ☐ **No disponible** ☐

EN CASO AFIRMATIVO, POR FAVOR, ENVIE UNA COPIA DE LOS RESULTADOS A EXPERIOR.

Prueba	Fecha (dd/mmm/aaaa)	Resultado	Unidades (si procede)

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Código de Protocolo		Número Centro		Número Paciente	
Número de referencia local (A cumplimentar por Experior)					

SECCIÓN 6.2.- TRATAMIENTO para el ACONTECIMIENTO ADVERSO.- Indique el(los) tratamiento(s) administrado(s) para el(los) acontecimiento(s) adverso(s). No anote medicación concomitante en este apartado.

Tratamiento administrado	Dosis (unidades)	Pauta	Vía de admin.	Fechas (dd/mmm/aaaa)		Indicación
				Inicio	fin	

SECCIÓN 7.- HISTORIA CLINICA y OTROS FACTORES DE RIESGO.- Por favor, describa aquellas condiciones médicas relevantes para el acontecimiento adverso: alergias previas, antecedentes familiares, personales, tabaco, alcoholismo,...

SECCIÓN 8.- OTRA INFORMACIÓN RELEVANTE.

¿Fue hospitalizado el paciente?	Sí ☐	No ☐	No procede ☐	No disponible ☐
En caso de ingreso y siempre que sea posible, por favor, facilite el informe de alta.	Fecha de ingreso: (dd/mmm/aaaa) ☐☐/☐☐☐/☐☐☐☐		Fecha de alta: (dd/mmm/aaaa) ☐☐/☐☐☐/☐☐☐☐	
¿Se trata de una sobredosis del producto en investigación?	Sí ☐	No ☐	No procede ☐	No disponible ☐
	En caso afirmativo:		**Intencionada** ☐	**Accidental** ☐
¿Se trata de un embarazo?	Sí ☐	No ☐	No procede ☐	No disponible ☐
	En caso afirmativo:		**Paciente** ☐	**Pareja** ☐

Sección 8.1.- FALLECIMIENTO.-Completar únicamente en caso de fallecimiento.

Fecha de la muerte (dd/mmm/aaaa)	
Especifique la causa de la muerte	
¿Se realizó autopsia?	Sí ☐ No ☐ No procede ☐ No disponible ☐ En caso afirmativo, por favor, envíe una copia del informe de autopsia a Experior.

POR FAVOR, ENVÍE <u>URGENTEMENTE</u> A FARMACOVIGILANCIA de EXPERIOR
POR FAX al 961.452.191; ó POR E-MAIL: farmacovigilancia@experior.es

Código de Protocolo		Número Centro		Número Paciente	
Número de referencia local (A cumplimentar por Experior)					

SECCIÓN 9.- INFORMACIÓN ADICIONAL/ SEGUIMIENTO DEL ACONTECIMIENTO ADVERSO.-(Por favor, proporcione cualquier información que considere relevante para la evaluación del Acontecimiento Adverso. Adjunte en este espacio la información de seguimiento, indicando únicamente datos nuevos).

SECCIÓN 10.- DATOS DEL CENTRO Y DEL INVESTIGADOR.

Nombre del investigador:		
Firma del investigador y fecha (dd/mmm/aaaa):		□□/□□□/□□□□
Dirección del centro, nº de teléfono y fax:		
Nombre de la persona del centro que notifica el(los) acontecimiento(s) (en caso que sea diferente al investigador principal)		
Fecha de notificación a Experior (dd/mmm/aaaa):	□□/□□□/□□□□	

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INSTRUCCIONES GENERALES

- La información recogida en este formulario permite realizar una evaluación del acontecimiento adverso, por lo que se ruega documentarlo lo mejor posible utilizando toda la información que haya disponible.
- El formulario debe ser completado en **MAYÚSCULAS** y con BOLÍGRAFO NEGRO.
- Cuando se desconozca o todavía no esté disponible la información solicitada, por favor, no deje la sección en blanco, indique **NO DISPONIBLE (N/D)**
- Cuando la información solicitada no sea relevante para el acontecimiento adverso que se está notificando, por favor, no deje la sección en blanco, indique **NO APLICABLE (N/A).**
- Siempre que sea posible, adjunte el **informe de alta** de hospitalización.
- **NO OLVIDE INCLUIR FECHA DE NOTIFICACIÓN Y FIRMA EN LA ÚLTIMA HOJA DEL FORMULARIO**.
- Se rellenará un ÚNICO formulario de notificación de Acontecimientos Adversos (con tantas hojas y documentación complementaria como sea necesario) por cada Acontecimiento Adverso. En caso de obtener **información de seguimiento,** la información adicional deberá ser cumplimentada en el formulario rellenado inicialmente, firmando y fechando la última hoja del formulario. En caso de que la nueva información implique una corrección de los datos aportados inicialmente, se tachará la información incorrecta, firmando y fechando al lado de dicha corrección tantas veces como correcciones y/o tachones se lleven a cabo en el formulario.
- Utilice tantas copias de la última hoja como necesite para describir toda la información que considere relevante para la evaluación del acontecimiento adverso.
- La última hoja debe firmarse y fecharse de nuevo en caso de notificaciones de seguimiento.
- No olvide establecer la **relación de causalidad** con el producto en investigación. A continuación se definen los criterios que puede elegir para establecer dicha relación:

➢ **Definitiva:** El Acontecimiento Adverso sigue una secuencia temporal razonable a partir del momento de la administración del fármaco y sigue un patrón de respuesta conocida del fármaco en estudio y no puede ser explicado razonablemente por otros factores tales como el estado clínico del paciente u otras intervenciones terapéuticas o medicaciones concomitantes administradas al paciente y, además, uno o más de los siguientes: a) aparece inmediatamente después de la administración del fármaco; b) mejora al suspender la administración del fármaco; c) reaparece a la reintroducción del fármaco.

➢ **Probable:** El Acontecimiento Adverso sigue una secuencia temporal razonable a partir del momento de la administración del fármaco y sigue un patrón de respuesta conocida del fármaco en estudio y no puede ser explicado razonablemente por otros factores tales como el estado clínico del paciente u otras intervenciones terapéuticas o medicaciones concomitantes administradas al paciente.

➢ **Posible:** El Acontecimiento Adverso sigue una secuencia temporal razonable a partir del momento de la administración del fármaco y/o sigue un patrón de respuesta conocida del fármaco en estudio, aunque podría ser producida por otros factores tales como el estado clínico del paciente u otras intervenciones terapéuticas o medicaciones concomitantes administradas al paciente.

➢ **Improbable:** El Acontecimiento Adverso es más probable que esté producido por otros factores tales como el estado clínico del paciente, intervenciones terapéuticas o medicaciones concomitantes administradas al paciente y no sigue un patrón de respuesta conocida del fármaco en estudio.

➢ **No Relacionado**: El Acontecimiento Adverso está claramente relacionado con otros factores tales como el estado clínico del paciente, intervenciones terapéuticas o medicaciones concomitantes administradas al paciente.

- La sección 3.4 permite explicar y describir la razón por la que considera que el acontecimiento adverso podría estar relacionado con la medicación en estudio. En caso de que se hayan suministrado más de dos especialidades farmacéuticas distintas, por favor, indique con cuál de ellas (con ambas, con ninguna) considera que podría estar relacionado el acontecimiento adverso.
- La sección 5, medicación concomitante hace referencia a aquella medicación que podría haber influido y/o contribuido al acontecimiento adverso. No es necesario que describa toda la medicación actual del paciente, sino solamente la que considere relevante para el acontecimiento adverso.
- La sección 6 permite enumerar las pruebas realizadas durante el diagnostico, así como, el tratamiento suministrado para resolver y/o estabilizar el acontecimiento adverso. Por favor, no dude en utilizar la sección 9, para detallar los resultados de dichas pruebas, o bien, utilice tantas páginas de seguimiento (sección 9) como necesite.
- La sección 7, historia clínica y otros factores, hace referencia a aquellas patologías o antecedentes personales del paciente que pueden haber influido o contribuido a la aparición del acontecimiento adverso. No es necesario que enumere todas las patologías del paciente, únicamente describa aquellas significativas y que hayan podido contribuir a la aparición del efecto adverso (ej alergias, alcoholismo en casos de hepatitis,..)
- En la sección 8, la opción Pareja debe ser señalada cuando la pareja del paciente que está participando en el ensayo clínico ha quedado embarazada. Se realizará un seguimiento durante el embarazo hasta que este llegue a término documentando cualquier patología acontecida en la madre, los detalles del parto y del recién nacido.
- En caso de fallecimiento, por favor, intente conseguir el informe de autopsia, si no es posible, describa con máximo detalle causa y hora de la muerte.

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