Clinical response to biologics for severe asthma: any relevance for sex in different age ranges?

Benoni Roberto, Panunzi Silvia, Batani Veronica, Moretti Francesca, Fuggini Stefano, Todesco Mattia, Senna Gianenrico, Poli Albino, Vianello Andrea, Caminati Marco

Please cite this article as: Roberto B, Silvia P, Veronica B, et al. Clinical response to biologics for severe asthma: any relevance for sex in different age ranges?. ERJ Open Res 2022; in press (https://doi.org/10.1183/23120541.00670-2021).

This manuscript has recently been accepted for publication in the ERJ Open Research. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

Copyright ©The authors 2022. This version is distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0. For commercial reproduction rights and permissions contact permissions@ersnet.org
Clinical response to biologics for severe asthma: any relevance for sex in different age ranges?

Benoni Roberto¹, Panunzi Silvia², Batani Veronica³, Moretti Francesca¹, Fuggini Stefano¹, Todesco Mattia⁴, Senna Gianenrico³,⁴, Poli Albino¹, Vianello Andrea⁵, Caminati Marco³

Affiliations:
1. Department of Diagnostics and Public Health, Section of Hygiene, University of Verona, Verona, Italy
2. Department of Diagnostics and Public Health, Unit of Epidemiology and Medical Statistics, University of Verona, Verona, Italy
3. Department of Medicine, University of Verona, Verona, Italy
4. Asthma Center and Allergy Unit, Verona University Hospital, Verona, Italy
5. Department of Cardiological, Thoracic and Vascular Sciences, Respiratory Pathophysiology Unit, University of Padua, Italy

Corresponding author:
Veronica Batani
Medical Doctor
Department of Medicine, University of Verona,
p.le A. Scuro, 10 – 37134, Verona, Italy
ve.batani@gmail.com
Whether sex can influence the clinical response to biologic treatment in severe asthma patients has not been fully addressed. The aim of this study was to investigate in severe asthma patients undergoing biologic treatment the individual evolution of lung function measurements and patient reported asthma control scores over a twelve-months follow-up period, in relation to patients’ sex, in different age ranges. Secondly, the change in the administered dose of oral corticosteroids (OCS) before and after twelve-months treatment was investigated. Overall 64 patients (58% females and 42% males) with a median age of 52 years were enrolled in the study. No relevant differences in terms of lung function, patient reported asthma control, exacerbation rate and daily OCS dose were observed by gender within the study timeframe. A separate sub-analysis by biologic treatment, confirmed the same finding. Stratifying individuals by age, we showed that elderly men resulted in lower lung function parameters’ values (FEV1% predicted and FEV1/FVC index) when compared to elderly women, whereas an opposite trend was observed in terms of ACT score. No other relevant differences were detected after age-stratification. According to our findings gender seems not to act as a determinant of treatment response to biologics in severe asthmatics. Although to be confirmed in larger studies, our data suggest that neither gender nor age of patients should limit the biologic treatment prescription, once that the eligibility criteria for that therapy are satisfied.
1. Introduction

Bronchial asthma affects about 4.5% of the general population, with not negligible differences according to countries, age ranges and sex. In adults, asthma is more prevalent in women whilst in the younger individuals more boys than girls are reported to be asthmatics. On the opposite, no relevant differences by sex can be observed after menopausal period[1-5]. Overall, less than 5% of asthmatics suffer from severe disease, according to the ERS/ATS definition [6-8]. Data from severe asthma registries show higher disease prevalence in women. According to the “Severe Asthma Network in Italy” 61.8% of Italian patients with severe asthma are female, characterised by more late onset disease, more comorbidities and poorer asthma control[4]. The complex interplay between hormonal fluctuations, inflammatory mechanisms and environmental factors may account for the sex differences, which still represent a controversial issue. Also, whether sex can influence the response to biologic treatment in patients with severe asthma is not completely clear. Up to now the evidence on the topic mainly comes from studies analyzing potential predictors of response to biologics, including sex, which does not seem to have any relevance [9-12]. However sex has not been primarily explored as a factor associated with the clinical response to biologic treatment in severe asthmatics so far.

The aim of the present study is to investigate in severe asthma patients the individual evolution of lung function measurements and patient reported asthma control scores over a twelve-months follow-up period of biological treatment, in relation to patients’ sex, overall and in different age ranges. Secondly, the change in the administered dose of oral corticosteroids (OCS) before and after twelve-months treatment was investigated.
2. Methods

2.1 Study subjects

Study population was sampled from the registry of the Interdisciplinary Network for the management of severe asthma in Veneto region, Italy, a non-profit collaboration project including Allergy and Respiratory Referral Centres for Severe Asthma located in the Northeast of Italy and approved by the local ethics committee [13,14]. The registry collects real-life but standardised clinical and functional information from adult patients who meet the following criteria: - A confirmed diagnosis of severe asthma according to the ERS/ATS definition [7]; - Eligibility to at least one of the biologic drugs currently marketed in Italy for severe asthma, according to the prescription requirements established by the Italian Regulatory Agency [15]. Patients diagnosed with Allergic Bronchopulmonary Aspergillosis and Eosinophilic Granulomatosis with Polyangiitis are excluded. For the present study all consecutive patients referring to Verona Centre and on biologic treatment for at least twelve months at the time of the analysis were considered.

2.2 Study design

In order to explore how sex, overall and in different age ranges, may influence a different response to biological therapies in patients with severe asthma a retrospective analysis was carried out, using data collected from the patients included into the registry, prescribed with biologic therapy for severe asthma and regularly followed-up at our Center. Clinical and functional data from visits at one, three, six, twelve months after the treatment start were collected and analysed.

2.3 Data collection

For each subject the following data were included in the analysis: age at the biologic treatment start, sex, body mass index (BMI), blood eosinophils counts (expressed as number of cells/mm3), nitric oxide (NO) exhaled fraction (expressed as parts per billion), smoking history, number of comorbidities, prescribed biologic drug, Asthma Control Test (ACT) measurements, FEV1% predicted FEV1/FVC % (Tiffeneau index) at baseline and at each visit time (one, three, six, twelve months after the treatment initiation). Tiffeneau
index is a validated measure of bronchial obstruction derived from the forced expiratory volume in one second and the forced vital capacity ratio (FEV1/FVC); it is used to differentiate between obstructive (in which both FEV1 and FEV1/FVC are reduced) and restrictive lung disease (in which FEV1 is reduced but FEV1/FVC is normal due to the contemporary reduction of both FEV1 and FVC). Normal Tiffeneau values are above >75-80% in adults and >90% in children. Values < 80% are suggestive for obstructive respiratory disease and lower values are related to a greater degree of obstruction [16]. The oral corticosteroids dose at baseline (mg of prednisone or equivalent/day) and post 12-months treatment as referred by the patient was analysed.

Regarding comorbidities, data were collected as follows: smoke history was considered positive in the case of current or former smoker patients; polyposis was recorded in the presence of a documented rhinoscopy or facial CT scan; bronchiectasis and interstitial lung diseases were included if confirmed by a high resolution lung CT scan; rhinitis was defined as patients’ referred diagnosis; atopy was defined as the detection of positive prick test and or serum specific IgE to aero-allergens; autoimmune diseases, dermatitis and Gastro-Esophageal Reflux Disease (GERD) were investigated among the medical records provided by the patients at the time of first assessment at the Referral Centre for severe asthma.

2.4. Statistical Analysis

Individual lung function measurements and ACT scores were modelled using Linear mixed models (LMMs) for longitudinal data and subject-specific random effects [17], in order to estimate individual and mean evolutions of FEV1% predicted, Tiffeneau% and ACT values for patients over time of follow-up and to compare predicted trajectories between males and females of different age, when adjusting for baseline clinical and demographic characteristics.

A first descriptive analysis was used to explore patients’ variables distribution at baseline (start of therapy) in the overall sample and separately for males, females and between age groups (tertiles of age in the sample under study).

For the first set of analyses, three random intercept LMMs models were fitted for each of the outcome and for each sex subgroup separately, including measurement time as fixed factor variable (with levels set up to one, three, six, twelve months after start of therapy). These models allowed for a direct interpretation of
fitted model parameters as representing the average longitudinal growth/decline of the lung function outcome over the follow-up time, distinctly for male and females [18].

In a second set of analyses, the cohort sample was investigated. Natural cubic B-splines [19] were used to parameterize nonlinear trajectories of the outcomes over time, for the overall sample of individuals. Natural cubic B-splines were chosen over standard cubic B-splines to improve the stability of the results-in particular beyond the boundary knots (baseline time and one year of follow-up time), two inner knots were chosen for FEV1% predicted and Tiffeneau% at one and six months after start of therapy, whether an additional knot was fitted at three months for ACT outcomes. The Akaike information criterion (AIC) and visualisations of the raw data and residuals were used to determine the preferred knot placement. Three-way interactions between sex, age and time of follow-up were tested and assumed in the three outcomes models. Using natural cubic B-splines allowed for modelling plausible non-linear growth over months of follow-up, but lack of direct interpretation of each of the estimated parameters. These models were therefore used to provide age-specific estimates of outcome measures over time, and graphically depict the association but sex and age and FEV1% predicted, Tiffeneau% and ACT growth/decline in our observed data, through the use of estimated marginal means plots.

To account for the heterogeneity in longitudinal evolutions between individuals LMMs were fitted to include individual-specific random slopes. The inclusion of random slopes was also tested. We considered both an unstructured correlation structure (assuming each variance and covariance is unique) and a first-order continuous autoregressive correlation structure (assuming measures closer in age are more correlated than measures more distant). ACF residual plots were used to determine the correlation structure; a first-order autoregressive (AR(1)) correlation structure was chosen in the final models. A weighted variance structure was added to model homogeneity of residuals at each visit time, in the LMM for ACT.

Point estimates and approximate 95% confidence intervals were estimated to be around the predicted mean growth for each outcome for each month of follow-up, sex group and along with three representative ages (35, 50, 70) years were the means of each age tertile group in the sample). Model parameters from the three-way interaction of follow-up cubic splines, sex and age are complex to interpret directly therefore predicted time-specific estimates were graphically plotted for the associations between
individuals’ sex and age and the evolution of the outcomes over months of follow-up. Models were adjusted for type of therapy undertaken, history of smoke status, number of comorbidities, body mass index, blood eosinophilia and NO exhaled fraction (FeNO) at baseline screening time. We deleted missing data on adjustment variables listwise.

Statistical analyses were performed in R 4.0.3, using additional packages nlme [20], splines [21], ggplot2 [22] and ggeffects [23] to produce the growth charts. P-values < 0.05 were considered as significant.

3. Results

3.1 Patients’ characteristics at baseline

Overall 64 patients were selected according to the inclusion criteria, representing 22.94% of patients included in the Network for the management of severe asthma in Veneto region, Italy, at the time of the analysis. Patients characteristics at baseline were reported for the whole sample and stratifying by sex and age subgroups (Table 1 and Table S1). Overall 37 patients (58%) were females and 27 (42%) were males, median age was 52 years and median bmi was 24 kg/m². The majority of the patients (79%) had no smoking history (88% among females and 68% among males)). Only a small percentage of patients had bronchiectasis (7%, 13% of female and no one among males), autoimmune diseases (5%), interstitial lung disease (3%), dermatitis (3%), gastroesophageal reflux disease (GERD) (19%); to the contrast, the majority of the patients had rhinitis (93%) and atopy (85%). Half of males did not take oral corticosteroids at baseline, whether the median OCSdose for females was 5 mg. Eosinophils counts were higher among females (650 mm³ in median) compared to males (435 mm³ in median). No relevant difference existed in general between sexes at baseline for lung function measurements (FEV1% predicted, FVC and ACT).

3.2 Outcomes measurements trends over visit time

Exploratory data analysis guided the model building process. Spaghetti plots (Figure 1) provided insight for raw individual’s data evolution, revealing a nonlinear trend of patients’ individual measures of FEV1%
predicted, Tiffeneau% and ACT during follow-up time distinguished by sex and age. Mean values of FEV1% predicted, Tiffeneau% and ACT at baseline and over follow-up visits for the overall sample were shown in Figure 2-4. LMMs with a fixed factor variable for follow-up time (four levels factor) were fitted for each outcome, in order to investigate the biological therapy responses, separately for the subset of males and females. The fitted models included age (years at start of therapy) as additional predictor. Model parameters provided an estimate of the average growth/decline in FEV1% predicted, Tiffeneau% and ACT values between the sex subgroups in each visit interval. A different evolution over time has resulted for the two subgroups (Table 2). A significant association between Tiffeneau% levels and age, was found for the females subgroup.

Considering the exploratory and the subgroup analysis results, in the overall sample analysis LMMs models were implemented choosing to include cubic splines functions, in order to parametrize individual trajectories over time of follow-up.

First, a model was fitted assuming interaction between sex and time of follow-up to investigate, resulting in no significant evidence for a different treatment response between the two groups of patients (Table S2). Secondly, a three-way interaction was assumed between sex, time of follow-up and patients’ age.

Estimated parameters for models with (Table S5) and without (Table S3) covariates adjustment, are provided.

The final (with covariate adjustment) models for three outcomes were used to estimate age-specific growth/decline curves for males and females, along with 95% CI (Table 3, Fig 2).

Estimated marginal mean predictions and plots were also presented for different age values (25,55,75) (Table S4, Fig S3).
3.3 Response to biological-drug therapy

**OCS**

The reduction in OCS daily dose at the twelve months follow-up in comparison with baseline has been explored. Among the 32 subjects regularly taking OCS at the time of biologic treatment start, OCS dose was reduced by the 82%, on average, from pre to post therapy. There was no clinically relevant difference in the reduction of OCS among males and females (females: mean 0.85 mg - median 1.00 mg [0.80 – 1.00]; male: mean 0.79 mg - median 1.00 mg [0.80 - 1.00]) and among patients’ age classes (19-47 years: mean 0.86 mg - median 1.00 mg [1.00-1.00]; 48-56 years: mean 0.82 mg - median 1.00 mg [0.90-1.00]; 57-83 years: mean 0.79 mg - median 1.00 mg [0.65-1.00]). OCS therapy was stopped by 23 patients (72%).

**FEV1% predicted**

*First exploration of separate lung function growth/decline rates for males and females evidenced FEV1% predicted improvement for both the subgroups, from start of therapy to end of follow-up (Table 2).* Females FEV1% predicted function was observed to improve at a higher rate after the first month since the start of therapy, while males improvement was evidenced at the highest rate after twelve months since the start of therapy. Age was not associated with different mean values of FEV1% predicted for females and males.

Whole cohort data were analysed in the final set of LMMs models (introduced in section 2.4), using natural cubic splines for non-linear growth/decline parameterization over time of follow-up (Table S5). It has been shown that young females, on average, reached a maximum improvement of FEV1% predicted after 3 months of therapy (from 77.4[66.1-88.7] to 86.3[75.0-97.5]), while for males FEV1% outcome was predicted to improve in the first 3 months (from 66.3[55.4-77.2] to 77.0 [66.2-87.9]), reaching a maximum value (77.6[66.6-88.6]), between 6 and 12 months after the start of biological-drug therapy (Fig 2-Table 3). Middle-aged males and females reached comparable mean predicted values of FEV1% at the end of the follow up period (Females = 79.1[71.6-86.6]; Males = 78.0[70.8-85.3]), with a comparable trend. Old males had an opposite trend in FEV1% predicted value when compared to females of the same age. Old males showed no increase in FEV1% outcome (from 81.8[68.7-94.8] at baseline to 78.7[65.5-91.8]) at the end of
follow up, while females - starting from lower values at baseline (62.3 [51.1-73.5]) - had an increase after one year of therapy (72.1[60.9-83.4]).

**Tiffeneau**

Significant improvement in Tiffeneau% values were evidenced from baseline to the end of follow-up for both males and females; the rate of improvement in females was higher at twelve months after start of therapy while males had a higher rate of improvement at one and three months, and a decline in the following visit times (Table 2). Females were also shown to have lower mean values of Tiffeneau% with increasing age whether this was not observed in males. From final LMMs models with natural cubic splines, for the whole cohort data, a reduction on Tiffeneau% function has been shown for females as their age increased. This was not observed in males. Over months of follow-up, mean predicted values for females were shown to increase from 92.0[85.2-98.8], 85.7[81.2-90.2], 77.3[70.6-84.0] respectively in the three age groups at t1 (one month after start of therapy) to 94.7[88.0-101.4], 88.8[84.3-93.2], 80.9[74.2-87.5] at t3 (three months after start of therapy). Values were shown to stay stable until the end of follow-up. Young and middle-aged males had the same increased in the first three months from 80.5[74.0-87.0] and 85.0[80.7-89.3] to 88.4[82.4-94.4] and 89.6[85.3-93.9] respectively. Predicted values decreased from the 6th to the 12th month. Old males had an improvement only in the first month and then decreased until the 12th month. At the end of follow-up young and middle-aged males showed higher values (95.1[88.3-102.0] and 89.2[84.6-93.7] respectively) as compared to baseline. Old males had lower values at 12 months (88.4[80.5-96.2]) when compared to baseline.

**ACT score**

Raw data exploration evidenced that ACT values, for both males and females in the cohort, had a decreasing variability along with follow-up (Fig. S1).

Significant improvement in ACT scores was evidenced at all visit times when exploring males and females growth rates separately, (Table 2). No association was found between age variable and ACT score for both the subgroups.
Final LMMs models with natural cubic splines, exploring the whole cohort data, showed for both females and males, regardless of age, a significant increase of mean predicted ACT scores from 17.5[15.8-19.2] and 17.6[15.6-19.5], respectively, to 23.3[22.6-24.1] and 23.9[23.2-24.6] at the end of the follow-up. Females had higher ACT scores with increasing age. This was not observed in males. Old females showed a first increase from baseline to the third month and a second increase from six to twelve months after the start of biological-drug therapy (Fig. 4).

4. Discussion
Our study described the trend of clinical and functional parameters in severe asthmatic males and females on biologic treatment over a 12 months follow up period. No significant differences in terms of lung function, patient reported asthma control, exacerbation rate and daily OCS dose were observed by gender within the study timeframe. When including age stratification, the lung function parameters (FEV 1 and Tiffeneau index) in elderly men were significantly lower than in elderly women. A similar trend could not be observed in terms of ACT score, which improved both in men and women in the same age range. No other significant differences were detected when stratifying by age.

When investigating the response to a biologic treatment for severe asthma, sex and age are usually considered as adjustment variables, and few studies have addressed them as determinants potentially impacting on the treatment effect. The available evidence on the topic mainly comes from studies analyzing potential predictors of response to biologics, including sex and age, which in that case do not seem to have any relevance [11, 24]. Only one study described female sex as a determinant of better response to omalizumab treatment in adults [25].

Our study aimed to explore as a primary outcome the potential interaction between sex, age and the response to biologic therapy over time. Of note, the statistical approach excluded all the confounding factors potentially impacting on treatment response, such as smoking history, BMI, comorbidities, baseline FeNO values and blood eosinophilia, in order to explore the “weight” of gender first, and age on biologic treatment clinical outcomes. As shown in Figure 2-4 an improvement in FEV1% predicted, Tiffeneau% and ACT scores was observed in both women and men, particularly one month after the start of biological
therapy. The subsequent time-points assessment revealed a less marked improvement, but still higher values in comparison with baseline could be observed.

A combined action of both biologic therapy and oral steroids, although tapered, may lead to a powerful early phase response. It can be assumed that once OCS daily intake is suspended or reduced to the minimal dose, the clinical control could be sustained by biologic therapy, and this might explain the minimal deflection in the clinical control. In fact, in the considered follow-up period, an 82% reduction in the oral steroid daily dose could be registered, regardless sex, and 72% of patients completely discontinued steroid therapy.

Although generally speaking, in clinical practice sex does not seem to act as a treatment response determinant, some sex and age related peculiarities deserve to be highlighted.

Considering the analysis for the two sex subsets separately (Table 2) asthmatic females experienced a faster increase in FEV1% predicted (being already significant at T1) that was maintained at each consecutive time point, while males seemed to have an improvement in the FEV1 % predicted that was evident only 12 months after the treatment start. This difference was better detailed in the analysis for the overall sample where a 3-way intersection has been parametrized between time, sex and age variables. In particular, elderly males seemed to have no improvement in FEV1% predicted over time. On the contrary, females of the same age had an improvement in FEV1% during follow-up visits (Figure 2, Table3). Similarly, elderly males showed no increase in the Tiffeneau% values, as compared to females of the same age.

For what concerned patient self-reported outcomes, ACT scores increased significantly in both males and females of all age classes at each time point. Only females showed significantly higher mean values as age increases. Comparable evolution of ACT scores can be observed over time (Table 3, Figure2), except for elderly females who showed a non-monotone ACT improvement.

Weak correlation between lung function and patient reported outcomes has already been described elsewhere[26]. Age and sex have been highlighted as determinants of asthma control perception [27]. In particular, discrepancies between lung function and ACT score seemed to be more relevant in the elderly. Distinguishing by sex, females have been described to be more sensitive to changes in lung function and to excessively amplify them when reporting subjective evaluation of asthma control, when compared to men.
Under the pathophysiological perspective, sex discrepancy in elderly may be related to the physiological sexual hormones variations that occur with aging. It is well-known that Th2-mediated airway inflammation is sustained and amplified by oestrogens (although the mechanisms are not completely clear) and down-regulated by androgens, which play a crucial protective role from type 2 bronchial inflammation [4,5,28,29]. In animal models testosterone demonstrated to modulate type 2 innate lymphoid cells proliferation and dehydroepiandrosterone (DHEA), a hormone upstream of testosterone, produced by the adrenals as well as by the gonads, showed to decrease serum eosinophils, IL-5, IL-4, and IFN-g levels [2]. In addition, it has been demonstrated that in men the above-mentioned sex hormones decline with age. That gradual and progressive longitudinal trend is the consequence of physiological changes in testicular secretory cells and in hypothalamic-pituitary sensitivity called andropause [30]. Free testosterone declined by 1.2%/year and DHEA by 3.1%/year with the incidence of hypogonadism increasing to about 20% of men over 60, 30% over 70 and 50% over 80 year of age [31-33]. On the other hand, with the onset of menopause, by the mid-sixth decade of life, all women experience a dramatic decline in oestrogens levels [30]. The above-mentioned evidence may provide a potential explanation to the lack of improvement or in some cases to the worsening in lung function, observed in older males after treatment with biologic drugs. In particular, the age-related reduction of androgens and of their protective role characterising andropause may account for the parallel increase with age of bronchial inflammation and for a limited response to therapeutic interventions. On the opposite improvement in treatment response in elderly women, could be explained by a reduction in underlying inflammation favoured by the drop in oestrogen in menopause.

The main limitations of our study are the retrospective study design and the small sample size. The investigated population was sampled from a severe asthma registry characterised by well-defined admission criteria that allows exploring a very specific group of asthma patients limiting the possible selection bias. On the other hand, this study is particularly powered by the use of models that have been using all the set of available patients measurements over visit times, giving not only a quantitative evaluation of the outcomes but also a qualitative estimation of their evolution over follow-up time.

To the best of our knowledge, no studies had analysed so far the relevance of sex and age in the clinical response to biologics for severe asthma as the primary outcome. Furthermore the “weight” of gender has
been explored by excluding potential confounding factors, such as the specific biologic treatment, history of smoke, BMI, number of comorbidities, baseline exhaled NO, and blood eosinophilia. However, the external validity of our findings needs to be confirmed in larger studies, also including more biologic drugs.

5. Conclusions
According to our findings gender seems not to act as a determinant of treatment response to biologics in severe asthmatics, in terms of lung function, patient reported outcomes, exacerbation rate and daily OCS dose, independently of the prescribed therapy (omalizumab or mepolizumab). However, when including age in the statistical model, elderly women showed a significantly higher lung function in comparison to men in the same age class, during the considered time window. No other significant differences were detected when stratifying by age. Although to be confirmed in larger studies our data suggest that neither sex or age should limit the biologic treatment prescription, once the eligibility criteria for that specific therapy are satisfied.

References

1. Schatz M, Camargo CA. The relationship of sex to asthma prevalence, health care utilization, and medications in a large managed care organization. Ann Allergy Asthma Immunol 2003; 91: 553–558.
2. Fuseini H, Newcomb DC. Mechanisms driving sex differences in asthma. Curr Allergy Asthma Rep 2017; 17: 19.
3. Shah R, Newcomb DC. Sex Bias in Asthma Prevalence and Pathogenesis. Front Immunol 2018; 9: 2997.
4. Senna G, Guerriero M, Paggiaro PL, Blasi F, Caminati M, Heffler E, Latorre M, Canonica GW, SANI. SANI-Severe Asthma Network in Italy: a way forward to monitor severe asthma. Clin Mol Allergy 2017; 15: 9.
5. Vink NM, Postma DS, Schouten JP, Rosmalen JGM, Boezen HM. Sex differences in asthma development and remission during transition through puberty: the TRacking Adolescents’ Individual Lives Survey (TRAILS) study. J Allergy Clin Immunol 2010; 126: 498-504.e1-6.
6. Wang E, Wechsler ME, Tran TN, Heaney LG, Jones RC, Menzies-Gow AN, Busby J, Jackson DJ, Pfeffer PE, Rhee CK, Cho YS, Canonica GW, Heffler E, Gibson PG, Hew M, Peters M, Harvey ES, Alacqua M, Zangrilli J, Bulathsinhala L, Carter VA, Chaudhry I, Eleangovan N, Hosseini N, Murray RB, Price DB. Characterization of Severe Asthma Worldwide: Data From the International Severe Asthma Registry. Chest 2020; 157: 790–804.
7. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet L-P, Brightling C, Chanez P, Dahlén S-E, Djukanovic R, Frey U, Gago M, Gibson P,
Hamid Q, Jajour NN, Mauad T, Sorkness RL, Teague WG. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014; 43: 343–373.
8. Vianello A, Caminati M, Andretta M, Menti AM, Tognella S, Senna G, Degli Esposti L. Prevalence of severe asthma according to the drug regulatory agency perspective: An Italian experience. World Allergy Organ J. 2019 May 10;12(4):100032.
9. Kavanagh JE, Green L, Fernandes M, Roxas C, Jackson DJ, Kent B, d’Ancona G. S79 Predictors of response to mepolizumab in oral corticosteroid dependent severe asthma. Thorax BMJ Publishing Group Ltd; 2018; 73: A49–A50.
10. Eger K, Kroes JA, Ten Brinke A, Bel EH. Long-Term Therapy Response to Anti-IL-5 Biologics in Severe Asthma- A Real-Life Evaluation. J Allergy Clin Immunol Pract 2021; 9: 1194–1200.
11. Casale TB, Busse W, Ziegler RS, Trzaskoma B, Yang M, Griffin NM, Chipps BE. Omalizumab Effectiveness by Biomarker Status in Patients with Asthma: Evidence From PROSPERO, A Prospective Real-World Study. The Journal of Allergy and Clinical Immunology: In Practice 2019; 7: 156-164.e1.
12. Silver J, Menzies-Gow A, Smith S, Mallett S, Bradford E, Yancey S, Albers F. BASELINE PERCENT PREDICTED FEV1 DOES NOT PREDICT A RESPONSE TO MEPOLIZUMAB IN PATIENTS WITH SEVERE EOSINOPHILIC ASThma: META-ANALYSIS FROM TWO PHASE 3 TRIALS. Chest 2019; 156: A459–A460.
13. Caminati M, Senna G, Chieco Bianchi F, Marchi MR, Vianello A, Micheletto C, Pomari C, Tognella S, Savoia F, Mirisola V, Rossi A; NEONET Study Group. Omalizumab management beyond clinical trials: the added value of a network model. Pulm Pharmacol Ther. 2014 Oct;29(1):74-9.
14. Caminati M, Cegolon L, Vianello A, Chieco Bianchi F, Festi G, Marchi MR, Micheletto C, Mazza F, Tognella S, Senna G. Mepolizumab for severe eosinophilic asthma: a real-world snapshot on clinical markers and timing of response. Expert Rev Respir Med. 2019 Dec;13(12):1205-1212.
15. https://www.aifa.gov.it
16. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Interpretative strategies for lung function tests. Eur Respir J. 2005 Nov;26(5):948-68.
17. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J of Statistical Software. 2015: 1 - 48.
18. Shepherd DA, Vos N, Reid SM, et al. Growth Trajectories in Genetic Subtypes of Prader-Willi Syndrome. Genes, 2020; .11(7), 736.
19. De Boor, De Boor C. A practical guide to splines. Vol. 27. New York: springer-verlag, 1978.
20. Pinheiro J, Bates D, DebRoy S, Sarkar D. R Development Core Team. nlme: Linear and Nonlinear Mixed Effects Models. R Package; R Foundation for Statistical Computing: Vienna, Austria, 2013.
21. R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing:Vienna, Austria, 2017.
22. Wickham H. ggplot2: Elegant Graphics for Data Analysis; Springer: New York, NY, USA, 2009
23. Lüdecke D. ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. Journal of Open Source Software, 2018: 3(26), 772.
24. Kroes JA, Zielhuis SW, van Roon EN, ten Brinke A. Prediction of response to biological treatment with monoclonal antibodies in severe asthma. Biochemical Pharmacology 2020; 179: 113978
25. Gibson PG, Reddel H, McDonald VM, Marks G, Jenkins C, Gillman A, Upham J, Sutherland M, Rimmer J, Thien F, Katsoulotos GP, Cook M, Yang I, Katelaris C, Bowler S, Langton D, Robinson P, Wright C, Yozghatlian V, Burgess S, Sivakumaran P, Jaffe A, Bowden J, Wark P a. B, Yan KY, Kritikos V, Peters M, Hew M, Aminazad A, Bint M, et al. Effectiveness and response predictors of omalizumab in a severe allergic asthma population with a high prevalence of comorbidities: the Australian Xolair Registry. Intern Med J 2016; 46: 1054–1062.
26. Ashton M Steele 1, Alicia E Meuret, Mark W Millard, Thomas Ritz. Discrepancies between lung function and asthma control: asthma perception and association with demographics and anxiety. Allergy Asthma Proc. Nov-Dec 2012;33(6):500-7.
27. So Young Park, Sun-Young Yoon, Bomi Shin, Hyouk-Soo Kwon 1, Tae-Bum Kim 1, Hee-Bom Moon 1, You Sook Cho 1. Clinical factors affecting discrepant correlation between asthma control test score and pulmonary function. Allergy Asthma Immunol Res. 2015 Jan;7(1):83–7.

28. Cephus J, Stier M, Fuseini H, Yung J, Toki S, Bloodworth M, Zhou W, Goleniewska K, Zhang J, Garon S, Hamilton R, Poloshuk V, Boyd K, Peebles R, Newcomb D. Testosterone attenuates group 2 innate lymphoid cell-mediated airway inflammation. Cell Rep 2017; 21: 2487–2499.

29. Laffont S, Blanquart E, Savignac M, Cénac C, Laverny G, Metzger D, Girard J-P, Belz GT, Pelletier L, Seillet C, Guéry J-C. Androgen signaling negatively controls group 2 innate lymphoid cells. J Exp Med 2017; 214: 1581–1592.

30. Chahal HS, Drake WM. The endocrine system and ageing. J Pathol 2007; 211: 173–180.

31. Gray A, Feldman HA, McKinlay JB, Longcope C. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. J Clin Endocrinol Metab 1991; 73: 1016–1025.

32. Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, Bremner WJ, McKinlay JB. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. J Clin Endocrinol Metab 2002; 87: 589–598.

33. Harman SM, Metter EJ, Tobin J, Pearson J, Blackman MR, Baltimore Longitudinal Study of Aging. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab 2001; 86: 724–731.
Table 1 - Baseline characteristics of patient with severe asthma distinguished by sex and age group (tertiles). Percentage and median with 25th-75th centiles intervals were used.

|                                         | Overall sample (n=64) | 19-47 years (n=13) | 48-56 years (n=14) | 57-83 years (n=10) | 19-47 years (n=9) | 48-56 years (n=6) | 57-83 years (n=12) | Total females (n=37) | Total males (n=27) |
|                                         |                        |                    |                    |                    |                  |                  |                    |                        |                   |
|                                         |                         |                    |                    |                    |                  |                  |                    |                        |                   |
| **Body mass index (Kg/m²)** (n=62; N/A=2) | Median                  | 24.25              | 22.82              | 22.0               | 25.8             | 22.2             | 25.1             | 26.55                  | 22.8              | 24.8              |
|                                         | (25th-75th centiles)    | (21.8-26.7)        | (19.9-25.8)        | (21.1-25.4)        | (21.9-26.5)      | (20.9-28.7)      | (23.7-25.6)      | (24.3-28.3)            | (21.0-26.5)        | (23.1-28.4)        |
| **Smoke history (n=58; N/A=6)**         | no                      | 46 (79%)           | 11 (85%)           | 10 (100%)          | 8 (80%)          | 6 (67%)          | 3 (50%)          | 8 (80%)                | 29 (88%)           | 17 (68%)           |
|                                         | yes                     | 12 (21%)           | 2 (15%)            | 0 (0%)             | 2 (20%)          | 3 (33%)          | 3 (50%)          | 2 (20%)                | 4 (12%)            | 8 (32%)            |
| **Number of comorbidities (n=64; N/A=0)** | Median                  | 3,0                | 3,0                | 2,0                | 3,0             | 3,0             | 2,0             | 3,0                    | 2,0                |                   |
|                                         | (25th-75th centiles)    | (2.0-3.0)          | (2.0-4.0)          | (1.0-3.0)          | (2.0-3.8)       | (2.0-3.0)       | (2.3-3.0)       | (1.8-3.0)              | (2.0-3.0)          | (2.0-3.0)          |
| **Polyposis (n=62; N/A=2)**             | no                      | 28 (45%)           | 6 (46%)            | 5 (39%)            | 6 (67%)         | 4 (44%)         | 1 (17%)         | 6 (50%)                | 17 (49%)           | 11 (41%)           |
|                                         | yes                     | 34 (55%)           | 7 (54%)            | 8 (62%)            | 3 (33%)         | 5 (56%)         | 5 (83%)         | 6 (50%)                | 18 (51%)           | 16 (59%)           |
| **Bronchiectasis (n=57; N/A=7)**        | no                      | 53 (93%)           | 10 (83%)           | 8 (80%)            | 10 (100%)       | 9 (100%)        | 6 (100%)        | 10 (100%)              | 28 (88%)           | 25 (100%)          |
|                                         | yes                     | 4 (7%)             | 2 (17%)            | 2 (20%)            | 0 (0%)          | 0 (0%)          | 0 (0%)          | 0 (0%)                 | 4 (13%)            | 0 (0%)             |
| **Rhinitis (n=58; N/A=6)**              | no                      | 4 (7%)             | 0 (0%)             | 1 (10%)            | 1 (10%)         | 0 (0%)          | 0 (0%)          | 2 (20%)                | 2 (6%)             | 2 (8%)             |
|                                         | yes                     | 54 (93%)           | 13 (100%)          | 9 (90%)            | 9 (100%)        | 6 (100%)        | 8 (80%)         | 31 (94%)               | 23 (92%)           |                   |
| **Atopy (n=59; N/A=5)**                 | no                      | 9 (15%)            | 0 (0%)             | 4 (40%)            | 2 (20%)         | 0 (0%)          | 1 (17%)         | 2 (18%)                | 6 (18%)            | 3 (12%)            |
|                                         | yes                     | 50 (85%)           | 13 (100%)          | 9 (90%)            | 9 (100%)        | 6 (100%)        | 8 (80%)         | 30 (96%)               | 20 (77%)           |                   |
|                      | yes | 50 (85%) | 13 (100%) | 6 (60%) | 8 (80%) | 9 (100%) | 5 (83%) | 9 (82%) | 27 (82%) | 23 (89%) |
|----------------------|-----|----------|-----------|---------|---------|----------|---------|---------|----------|----------|
| Autoimmune diseases  | no  | 55 (95%) | 12 (92%)  | 9 (90%) | 9 (100%)| 6 (100%) | 10 (100%)| 30 (91%)| 25 (100%)|          |
|                      | yes | 3 (5%)   | 1 (8%)    | 1 (10%) | 0 (0%)  | 0 (0%)   | 0 (0%)  | 3 (9%)  | 0 (0%)   |          |
| Interstitial lung    | no  | 56 (97%) | 12 (92%)  | 9 (90%) | 10 (100%)| 9 (100%) | 6 (100%)| 10 (100%)| 31 (94%) | 25 (100%)|
| disease              | yes | 2 (3%)   | 1 (8%)    | 0 (0%)  | 1 (10%) | 0 (0%)   | 0 (0%)  | 2 (6%)  | 0 (0%)   |          |
| Dermatitis           | no  | 56 (97%) | 12 (92%)  | 10 (100%)| 9 (100%)| 6 (100%) | 10 (100%)| 31 (94%)| 25 (100%)|          |
|                      | yes | 2 (3%)   | 1 (8%)    | 0 (0%)  | 1 (10%) | 0 (0%)   | 0 (0%)  | 2 (6%)  | 0 (0%)   |          |
| Gastro-Esophageal    | no  | 47 (81%) | 10 (77%)  | 9 (90%) | 5 (50%) | 8 (89%) | 6 (100%)| 9 (90%) | 24 (73%) | 23 (92%) |
| Reflux Disease (GERD)| yes | 11 (19%) | 3 (23%)   | 1 (10%) | 5 (50%) | 1 (11%) | 0 (0%) | 1.10 (0%)| 9 (27%)  | 2 (8%)   |
| Biologic drugs       | Mepolizumab | 26 (43%) | 2 (15%) | 10 (83%)| 4 (40%) | 2 (22%) | 2 (33%) | 6 (55%) | 16 (46%) | 10 (39%) |
|                      | Omalizumab  | 35 (57%) | 11 (85%) | 2 (17%) | 5 (60%) | 7 (78%) | 4 (67%) | 5 (46%) | 19 (54%) | 16 (62%) |
| Oral corticosteroids | Median 2.5 | 50 | 0 | 2.5 | 0 | 2.5 | 5 | 0 | 5 |
| (mg)                 | (25th-75th centiles) | (0.0-7.2) | (0.0-5.0) | (0.0-5.0) | (0.0-10.0) | (0.0-5.0) | (0.0-5.0) | (3.8-12.5) | (0.0-6.3) | (0.0-10.0) |
| Blood eosinophils    | Median 570 | 720 | 880 | 340 | 500 | 420 | 400 | 650 | 435 |
| (cells/mm³)          | (n=61-N/A=3) |
| Metric             | Median | Lower Quartile | Upper Quartile | Lower Limit | Upper Limit |
|--------------------|--------|----------------|----------------|-------------|-------------|
| **(25th-75th centiles)** |         |                |                |             |             |
| Exhaled NO (ppb)   | 48.2   | 37.12          | 74.0           | 39.66       | 41.4        |
|                      | 41.4   | 59.0           | 55.4           | 49.0        | 45.0        |
| Fev1 % t0           | 70.0   | 85.0           | 64.5           | 69.0        | 76.0        |
|                      | 69.0   | 77.0           | 77.0           | 68.0        | 73.5        |
| FVC % t0            | 86.0   | 88.0           | 75.5           | 83.0        | 92.0        |
|                      | 83.0   | 85.0           | 88.0           | 88.0        | 84.0        |
| Tiffeanu % t0       | 87.5   | 97.0           | 82.0           | 78.5        | 83.0        |
|                      | 83.0   | 91.0           | 83.0           | 91.0        | 87.5        |
| ACT t0              | 17.0   | 15.0           | 17.0           | 18.5        | 18.0        |
|                      | 18.0   | 16.0           | 18.0           | 16.0        | 17.0        |
| **(25th-75th centiles)** |         |                |                |             |             |
|                      | 15.0-19.0 | 13.0-17.0     | 15.0-19.0      | 17.0-24.0   | 15.0-18.0   |
|                      | 14.5-20.5 | 16.0-20.5     | 14.8-19.0      | 15.2-19.0   |             |
Table 2 – Estimated parameters for LMMs with linear splines fitted on the subset of females and males separately.

|                | Females (n=37; n° obs=179) | Males (n=27; n° obs=129) |
|----------------|-----------------------------|--------------------------|
|                | Value | Std. Error | p-value | Value | Std. Error | p-value |
| FEV 1 percentage |       |            |         |       |            |         |
| (Intercept)    | 88.84 | 13.29      | <0.001  | 70.60 | 11.31      | <0.001  |
| t1             | 5.27  | 1.95       | 0.008   | 3.47  | 2.02       | 0.090   |
| t3             | 8.02  | 2.39       | 0.001   | 2.44  | 2.30       | 0.290   |
| t6             | 7.89  | 2.58       | 0.003   | 1.84  | 2.33       | 0.432   |
| t12            | 7.36  | 2.69       | 0.007   | 5.24  | 2.37       | 0.029   |
| age            | -0.36 | 0.25       | 0.162   | 0.06  | 0.21       | 0.796   |
| Tiffeneau percentage |       |            |         |       |            |         |
| (Intercept)    | 112.08| 7.16       | <0.001  | 83.15 | 7.81       | <0.001  |
| t1             | 1.50  | 1.11       | 0.178   | 3.54  | 1.34       | 0.010   |
| t3             | 2.58  | 1.32       | 0.052   | 4.04  | 1.53       | 0.009   |
| t6             | 2.69  | 1.39       | 0.055   | 1.67  | 1.58       | 0.292   |
| t12            | 3.45  | 1.45       | 0.019   | 2.13  | 1.61       | 0.188   |
| age            | -0.48 | 0.14       | 0.001   | 0.04  | 0.15       | 0.766   |
| ACT score      |       |            |         |       |            |         |
| (Intercept)    | 14.45 | 1.53       | <0.001  | 17.70 | 1.33       | <0.001  |
| t1             | 4.10  | 0.69       | <0.001  | 3.96  | 0.72       | <0.001  |
| t3             | 5.14  | 0.80       | <0.001  | 5.64  | 0.76       | <0.001  |
| t6             | 5.62  | 0.77       | <0.001  | 5.68  | 0.77       | <0.001  |
| t12            | 5.98  | 0.75       | <0.001  | 6.20  | 0.76       | <0.001  |
| age            | 0.05  | 0.03       | 0.050   | 0.00  | 0.02       | 0.876   |
Table 3 – Predictive values of FEV1%, Tiffeneau% and ACT for males and females of 35, 50 and 70 years old. Linear mixed models with interaction between months after start of therapy (natural cubic splines functions), sex and age. Models were also adjusted for: biological-drug, history of smoke, BMI, number of comorbidities, exhaled NO, and blood eosinophilia, held constant at their mean values or at their category’s proportions in case of factors.

| Age: 35 years | Females | | Males | |
|---------------|---------|---------|-------|---|
| **FEV 1 percentage** | | | | |
| t0 | 77.41 | [66.09 - 88.73] | 66.27 | [55.36 - 77.17] |
| t1 | 82.10 | [71.26 - 92.94] | 71.78 | [61.39 - 82.17] |
| t3 | 86.25 | [75.02 - 97.48] | 77.03 | [66.17 - 87.89] |
| t6 | 84.79 | [73.41 - 77.17] | 76.36 | [65.39 - 87.33] |
| t12 | 84.33 | [72.94 - 95.73] | 77.58 | [66.59 - 88.57] |
| **Tiffeneau percentage** | | | | |
| t0 | 62.33 | [51.13 - 73.53] | 61.79 | [50.97 - 72.61] |
| t1 | 67.29 | [56.64 - 77.94] | 79.19 | [66.73 - 91.64] |
| t3 | 73.65 | [62.56 - 84.74] | 75.36 | [63.43 - 88.29] |
| t6 | 76.20 | [64.93 - 87.48] | 73.27 | [60.15 - 86.39] |
| t12 | 72.13 | [60.85 - 83.42] | 78.66 | [65.52 - 91.79] |
| **ACT score*** | | | | |
| t0 | 91.98 | [85.2 - 98.77] | 80.48 | [73.97 - 87.00] |
| t1 | 93.13 | [86.58 - 99.68] | 84.59 | [78.34 - 90.84] |
| t3 | 94.67 | [87.95 - 101.4] | 88.43 | [81.99 - 94.88] |
| t6 | 95.50 | [88.67 - 101.0] | 87.54 | [80.97 - 94.10] |
| t12 | 95.13 | [88.28 - 101.97] | 86.19 | [79.62 - 92.77] |
| **Age: 50 years** | | | | |
| t0 | 85.71 | [81.21 - 90.21] | 85.03 | [80.73 - 89.33] |
| t1 | 87.15 | [82.79 - 91.51] | 87.92 | [83.8 - 92.05] |
| t3 | 88.76 | [84.29 - 93.22] | 89.63 | [85.38 - 93.88] |
| t6 | 89.09 | [84.56 - 93.62] | 86.83 | [82.49 - 91.16] |
| t12 | 89.16 | [84.63 - 93.70] | 87.12 | [82.78 - 91.46] |
| **Age: 70 years** | | | | |
| t0 | 77.34 | [70.63 - 84.04] | 91.09 | [83.26 - 98.91] |
| t1 | 79.18 | [72.74 - 85.61] | 92.37 | [84.84 - 99.90] |
| t3 | 80.86 | [74.23 - 87.50] | 91.22 | [83.48 - 98.97] |
| t6 | 80.54 | [73.78 - 87.29] | 85.88 | [78.01 - 93.76] |
| t12 | 81.21 | [74.44 - 87.97] | 88.35 | [80.47 - 96.24] |

**Adjusted for:** N comorbidity = 2.65, BMI = 24.5, exhaled NO = 61.7, Blood eosinophilia = 642.7; **Adjusted for:** N comorbidity = 2.65, BMI = 24.6, exhaled NO = 61.7, Blood eosinophilia = 654.7; ***Adjusted for:** N comorbidity = 2.63, BMI = 24.8, exhaled NO = 63.8, Blood eosinophilia = 654.8
### Table S1 - Baseline characteristics of patient with severe asthma distinguished by age group (tertiles). Percentage and median with interquartile range (IQR) were used.

|                     | Age group |   |   |   |
|---------------------|-----------|---|---|---|
|                     | 19-47 years | 48-56 years | 57-83 years |
| **BMI (Kg/m²)**     | Median (IQR) | 22.6 (20.0, 27.2) | 23.3 (21.5, 25.5) | 26.2 (24.2, 27.7) |
| History of smoke    | no | 17 (77.3) | 13 (81.2) | 16 (80.0) |
|                     | yes | 5 (22.7) | 3 (18.8) | 4 (20.0) |
| **N° comorbidity**  | Median (IQR) | 3.00 (2.00, 3.00) | 2.50 (1.75, 3.00) | 2.00 (2.00, 3.00) |
| Polyposis           | no | 10 (45.5) | 6 (31.6) | 12 (57.1) |
|                     | yes | 12 (54.5) | 13 (68.4) | 9 (42.9) |
| Bronchiectasis      | no | 19 (90.5) | 14 (87.5) | 20 (100.0) |
|                     | yes | 2 (9.5) | 2 (12.5) | 0 (0.0) |
| Rhinitis            | no | 0 (0.0) | 1 (6.2) | 3 (15.0) |
|                     | yes | 22 (100.0) | 15 (93.8) | 17 (85.0) |
| Atopy               | no | 0 (0.0) | 5 (31.2) | 4 (19.0) |
|                     | yes | 22 (100.0) | 11 (68.8) | 17 (81.0) |
| Autoimmune diseases | no | 21 (95.5) | 15 (93.8) | 19 (95.0) |
|                     | yes | 1 (4.5) | 1 (6.2) | 1 (5.0) |
| Interstitial lung disease | no | 21 (95.5) | 15 (93.8) | 20 (100.0) |
|                     | yes | 1 (4.5) | 1 (6.2) | 0 (0.0) |
| Dermatitis          | no | 21 (95.5) | 16 (100.0) | 19 (95.0) |
|                     | yes | 1 (4.5) | 0 (0.0) | 1 (5.0) |
| MRGE                | no | 18 (81.8) | 15 (93.8) | 14 (70.0) |
|                     | yes | 4 (18.2) | 1 (6.2) | 6 (30.0) |
| Biologic-drug therapy | Mepolizumab | 4 (18.2) | 12 (66.7) | 10 (47.6) |
|                     | Omalizumab | 18 (81.8) | 6 (33.3) | 11 (52.4) |
| OCS (mg)            | Median (IQR) | 0.00 (0.00, 5.00) | 0.00 (0.00, 5.00) | 5.00 (0.00, 11.88) |
| Exhaled NO (ppb)    | Median (IQR) | 38.4 (29.6, 65.5) | 73.0 (47.0, 97.6) | 46.5 (21.9, 86.6) |
| Blood eosinophilia (cells/mm³) | Median (IQR) | 645.0 (432.5, 862.5) | 740.0 (287.5, 1005.0) | 400.0 (260.0, 702.5) |
| FEV1 percentage     | Median (IQR) | 75.0 (63.5, 85.8) | 66.0 (49.5, 81.2) | 73.0 (62.0, 81.0) |
| FVC percentage      | Median (IQR) | 85.5 (68.2, 101.0) | 80.5 (68.5, 100.2) | 87.0 (83.0, 91.0) |
| Tiffeneau percentage | Median (IQR) | 91.5 (83.8, 98.8) | 82.0 (73.0, 89.0) | 88.0 (77.0, 93.0) |
| ACT score           | Median (IQR) | 16.0 (14.0, 18.0) | 16.0 (14.5, 20.0) | 18.0 (16.0, 21.0) |

Body Mass Index (BMI), Oral corticosteroids (OCS), Forced Expiratory Volume (FEV), Forced Vital Capacity (FVC), Asthma Control Test (ACT).
Figure S1 – Individual raw data trajectories (thin blue lines) and loess smoothed mean curves with confidence intervals (dark blue thick curve and shaded contours) of FEV1 percentage, Tiffeneau percentage and ACT score outcomes in the overall sample, before the start of biological-drug therapy (t0) and during the follow-up visits (1, 3, 6 and 12 months). Mean and standard deviation are shown in the table for the overall sample and grouping by sex of the participants.
Figure S2 – Predicted values (coloured lines) and confidence intervals (shaded contours) for FEV1% of females (pink) and males (light blue) in our study cohort, holding all the other variables in the model constant at their mean value (for numeric vectors) or at their factor’s category proportions (for factor variables).
### Table S2 – Estimated parameters of LMMs with natural cubic spline with interaction between time and sex for FEV1%, Tiffeneau% and ACT score.

#### Fev1 percentage

| Parameter                          | Value  | Std.Error | DF     | t-value | p-value |
|------------------------------------|--------|-----------|--------|---------|---------|
| (Intercept)                        | 78.91  | 9.21      | 238.00 | 8.56    | <0.001  |
| ns(time, knots = c(1, 6))1        | 3.14   | 3.36      | 238.00 | 0.93    | 0.351   |
| ns(time, knots = c(1, 6))2        | 15.24  | 3.91      | 238.00 | 3.89    | <0.001  |
| ns(time, knots = c(1, 6))3        | 1.58   | 2.28      | 238.00 | 0.70    | 0.487   |
| Sex(M)                             | 3.11   | 4.80      | 61.00  | 6.65    | 0.519   |
| age                                | -0.16  | 0.17      | 61.00  | -0.94   | 0.353   |
| ns(time, knots = c(1, 6))1:Sex(M)  | -5.53  | 5.18      | 238.00 | -1.07   | 0.287   |
| ns(time, knots = c(1, 6))2:Sex(M)  | -8.84  | 6.10      | 238.00 | -1.45   | 0.149   |
| ns(time, knots = c(1, 6))3:Sex(M)  | 0.76   | 3.51      | 238.00 | 0.22    | 0.828   |

#### Tiffeneau percentage

| Parameter                          | Value  | Std.Error | DF     | t-value | p-value |
|------------------------------------|--------|-----------|--------|---------|---------|
| (Intercept)                        | 99.35  | 5.67      | 238.00 | 17.52   | <0.001  |
| ns(time, knots = c(1, 6))1        | 1.20   | 2.00      | 238.00 | 0.60    | 0.548   |
| ns(time, knots = c(1, 6))2        | 5.63   | 2.30      | 238.00 | 2.45    | 0.015   |
| ns(time, knots = c(1, 6))3        | 1.69   | 1.33      | 238.00 | 1.27    | 0.205   |
| Sex(M)                             | -1.73  | 2.94      | 60.00  | -0.59   | 0.558   |
| age                                | -0.23  | 0.10      | 60.00  | -2.24   | 0.029   |
| ns(time, knots = c(1, 6))1:Sex(M)  | -4.50  | 3.08      | 238.00 | -1.46   | 0.146   |
| ns(time, knots = c(1, 6))2:Sex(M)  | 0.77   | 3.55      | 238.00 | 0.22    | 0.829   |
| ns(time, knots = c(1, 6))3:Sex(M)  | -3.41  | 2.04      | 238.00 | -1.67   | 0.096   |

#### ACT score

| Parameter                          | Value  | Std.Error | DF     | t-value | p-value |
|------------------------------------|--------|-----------|--------|---------|---------|
| (Intercept)                        | 15.69  | 1.16      | 200.00 | 13.56   | <0.001  |
| ns(time, knots = c(1, 3, 6))1      | 4.46   | 0.78      | 200.00 | 5.70    | <0.001  |
| ns(time, knots = c(1, 3, 6))2      | 4.21   | 0.66      | 200.00 | 6.38    | <0.001  |
| ns(time, knots = c(1, 3, 6))3      | 11.37  | 1.58      | 200.00 | 7.22    | <0.001  |
| ns(time, knots = c(1, 3, 6))4      | 2.82   | 0.40      | 200.00 | 7.01    | <0.001  |
| Sex(M)                             | 0.75   | 1.20      | 53.00  | 0.62    | 0.538   |
| age                                | 0.03   | 0.02      | 53.00  | 1.63    | 0.110   |
| ns(time, knots = c(1, 3, 6))1:Sex(M)| 1.02 | 1.24      | 200.00 | 0.83    | 0.410   |
| ns(time, knots = c(1, 3, 6))2:Sex(M)| -0.44| 1.04      | 200.00 | -0.42   | 0.673   |
| ns(time, knots = c(1, 3, 6))3:Sex(M)| -0.35| 2.50      | 200.00 | -0.14   | 0.890   |
| ns(time, knots = c(1, 3, 6))4:Sex(M)| 0.40| 0.64      | 200.00 | 0.62    | 0.534   |
Table S3 – Estimated parameters of LMMs with natural cubic spline with interaction between time, sex, and age for FEV1%, Tiffeneau% and ACT score

### FEV1 percentage*

| Estimate | Std.Error | DF  | t-value | p-value |
|----------|-----------|-----|---------|---------|
| Intercept | 93.35     | 13.46 | 232.00 | 6.94    | <0.001 |
| ns(time, knots = c(1, 6))1 | -12.75 | 14.29 | 232.00 | -0.89 | 0.370 |
| ns(time, knots = c(1, 6))2 | 4.31 | 16.54 | 232.00 | 0.26 | 0.790 |
| ns(time, knots = c(1, 6))3 | -3.62 | 9.39 | 232.00 | -0.39 | 0.700 |
| Sex(M) | -38.58 | 19.73 | 60.00 | -1.96 | 0.060 |
| age | -0.44 | 0.26 | 60.00 | -1.72 | 0.090 |
| ns(time, knots = c(1, 6))1:Sex(M) | 30.50 | 21.01 | 232.00 | 1.45 | 0.150 |
| ns(time, knots = c(1, 6))2:Sex(M) | 49.36 | 24.41 | 232.00 | 2.02 | 0.040 |
| ns(time, knots = c(1, 6))3:Sex(M) | 11.10 | 13.82 | 232.00 | 0.80 | 0.420 |
| ns(time, knots = c(1, 6))1:age | 0.31 | 0.27 | 232.00 | 1.14 | 0.260 |
| ns(time, knots = c(1, 6))2:age | 0.21 | 0.31 | 232.00 | 0.68 | 0.500 |
| ns(time, knots = c(1, 6))3:age | 0.10 | 0.18 | 232.00 | 0.57 | 0.570 |
| Sex(M):age | 0.82 | 0.37 | 60.00 | 2.18 | 0.030 |
| ns(time, knots = c(1, 6))1:Sex(M):age | -0.71 | 0.40 | 232.00 | -1.77 | 0.080 |
| ns(time, knots = c(1, 6))2:Sex(M):age | -1.14 | 0.46 | 232.00 | -2.46 | 0.010 |
| ns(time, knots = c(1, 6))3:Sex(M):age | -0.20 | 0.26 | 232.00 | -0.78 | 0.440 |

### Tiffeneau percentage**

| Estimate | Std.Error | DF  | t-value | p-value |
|----------|-----------|-----|---------|---------|
| Intercept | 112.99 | 7.92 | 232.00 | 14.27 | <0.001 |
| ns(time, knots = c(1, 6))1 | 5.80 | 8.47 | 232.00 | 0.68 | 0.490 |
| ns(time, knots = c(1, 6))2 | 2.69 | 9.57 | 232.00 | 0.28 | 0.780 |
| ns(time, knots = c(1, 6))3 | 1.29 | 5.40 | 232.00 | 0.24 | 0.810 |
| Sex(M) | -38.49 | 11.60 | 59.00 | -3.32 | <0.001 |
| age | -0.50 | 0.15 | 59.00 | -3.32 | <0.001 |
| ns(time, knots = c(1, 6))1:Sex(M) | 10.64 | 12.47 | 232.00 | 0.85 | 0.390 |
| ns(time, knots = c(1, 6))2:Sex(M) | 27.96 | 14.09 | 232.00 | 1.98 | 0.050 |
| ns(time, knots = c(1, 6))3:Sex(M) | 3.53 | 7.94 | 232.00 | 0.44 | 0.660 |
| ns(time, knots = c(1, 6))1:age | -0.09 | 0.16 | 232.00 | -0.56 | 0.580 |
| ns(time, knots = c(1, 6))2:age | 0.06 | 0.18 | 232.00 | 0.32 | 0.750 |
| ns(time, knots = c(1, 6))3:age | 0.01 | 0.10 | 232.00 | 0.08 | 0.930 |
| Sex(M):age | 0.72 | 0.22 | 59.00 | 3.27 | <0.001 |
| ns(time, knots = c(1, 6))1:Sex(M):age | -0.30 | 0.24 | 232.00 | -1.26 | 0.210 |
| ns(time, knots = c(1, 6))2:Sex(M):age | -0.54 | 0.27 | 232.00 | -2.00 | 0.050 |
| ns(time, knots = c(1, 6))3:Sex(M):age | -0.14 | 0.15 | 232.00 | -0.91 | 0.360 |

### ACT score***

| Estimate | Std.Error | DF  | t-value | p-value |
|----------|-----------|-----|---------|---------|
| Intercept | 10.56 | 3.19 | 192.00 | 3.30 | <0.001 |
| ns(time, knots = c(1, 3, 6))1 | 12.19 | 3.18 | 192.00 | 3.84 | <0.001 |
| ns(time, knots = c(1, 3, 6))2 | 2.24 | 2.69 | 192.00 | 0.83 | 0.410 |
| ns(time, knots = c(1, 3, 6))3 | 16.77 | 6.54 | 192.00 | 2.57 | 0.010 |
| ns(time, knots = c(1, 3, 6))4 | 5.03 | 1.70 | 192.00 | 2.97 | <0.001 |
| Sex(M) | 6.18 | 4.83 | 52.00 | 1.28 | 0.210 |
| age | 0.13 | 0.06 | 52.00 | 2.11 | 0.040 |
| ns(time, knots = c(1, 3, 6))1:Sex(M) | -8.16 | 4.80 | 192.00 | -1.70 | 0.090 |
| ns(time, knots = c(1, 3, 6))2:Sex(M) | 3.10 | 4.06 | 192.00 | 0.76 | 0.450 |
| ns(time, knots = c(1, 3, 6))3:Sex(M) | -3.74 | 9.89 | 192.00 | -0.38 | 0.710 |
| ns(time, knots = c(1, 3, 6))4:Sex(M) | -1.29 | 2.56 | 192.00 | -0.50 | 0.620 |
| ns(time, knots = c(1, 3, 6))1:age | -0.15 | 0.06 | 192.00 | -2.51 | 0.010 |
| ns(time, knots = c(1, 3, 6))2:age | 0.04 | 0.05 | 192.00 | 0.75 | 0.450 |
| ns(time, knots = c(1, 3, 6))3:age | -0.11 | 0.12 | 192.00 | -0.85 | 0.390 |
| ns(time, knots = c(1, 3, 6))4:age | -0.04 | 0.03 | 192.00 | -1.34 | 0.180 |
| Sex(M):age | -0.11 | 0.09 | 52.00 | -1.16 | 0.250 |
| ns(time, knots = c(1, 3, 6))1:Sex(M):age | 0.18 | 0.09 | 192.00 | 1.98 | 0.050 |
| ns(time, knots = c(1, 3, 6))2:Sex(M):age | -0.07 | 0.08 | 192.00 | -0.90 | 0.370 |
| ns(time, knots = c(1, 3, 6))3:Sex(M):age | 0.07 | 0.19 | 192.00 | 0.35 | 0.720 |
| ns(time, knots = c(1, 3, 6))4:Sex(M):age | 0.03 | 0.05 | 192.00 | 0.67 | 0.500 |

*Number of Observations: 308, Number of Groups: 64; **Number of Observations: 307, Number of Groups: 63; ***Number of Observations: 264, Number of Groups: 56
Table S4 – Predictive values of FEV1%, Tiffeneau% and ACT for males and females of 25, 55 and 75 years old. Linear mixed models with interaction between months after start of therapy (natural cubic splines functions), sex and age. Models were also adjusted for: biological-drug, history of smoke, BMI, number of comorbidities, exhaled NO, and blood eosinophilia, held constant at their mean values or at their category’s proportions in case of factors.

| Age: 25 years | Females | | Males | |
|---------------|---------|---|---|---|
| t0            | 81.72   | [66.37 - 97.06] | 61.83 | [46.4 - 77.26] |
| t1            | 86.33   | [71.68 - 100.98] | 69.67 | [54.96 - 84.37] |
| t3            | 89.85   | [74.64 - 105.06] | 77.5  | [62.15 - 92.86] |
| t6            | 87.24   | [71.81 - 77.26]  | 77.25 | [61.73 - 92.77] |
| t12           | 87.82   | [72.37 - 103.27] | 77.28 | [61.73 - 92.82] |

| Age: 55 years | Females | | Males | |
|---------------|---------|---|---|---|
| t0            | 68.79   | [61.34 - 76.25] | 75.14 | [67.46 - 82.81] |
| t1            | 73.64   | [66.48 - 80.8]  | 76.01 | [68.69 - 83.34] |
| t3            | 79.05   | [71.65 - 86.45] | 76.07 | [68.45 - 83.7]  |
| t6            | 79.88   | [72.39 - 87.38] | 74.6  | [66.87 - 82.32] |
| t12           | 77.36   | [69.86 - 84.86] | 78.2  | [70.46 - 85.93] |

| Age: 75 years | Females | | Males | |
|---------------|---------|---|---|---|
| t0            | 60.18   | [47.05 - 73.3]  | 84.01 | [68.61 - 99.4]  |
| t1            | 65.18   | [52.7 - 77.65]  | 80.25 | [65.55 - 94.95] |
| t3            | 71.85   | [58.85 - 84.85] | 75.12 | [59.86 - 90.38] |
| t6            | 74.98   | [61.76 - 88.19] | 72.83 | [57.34 - 88.32] |
| t12           | 70.39   | [57.16 - 83.62] | 78.81 | [63.31 - 94.31] |

| Tiffeneau percentage** | Females | | Males | |
|-------------------------|---------|---|---|---|
| Age: 25 years | Females | | Males | |
| t0            | 96.17   | [86.98 - 105.35] | 77.45 | [68.23 - 86.68] |
| t1            | 97.12   | [88.28 - 105.96] | 82.36 | [73.51 - 91.22] |
| t3            | 98.62   | [89.53 - 107.71] | 87.64 | [78.51 - 96.76] |
| t6            | 99.78   | [90.52 - 86.68]  | 88.01 | [78.71 - 97.3]  |
| t12           | 99.1    | [89.84 - 108.37] | 85.58 | [76.27 - 94.89] |

| Tiffeneau percentage** | Females | | Males | |
|-------------------------|---------|---|---|---|
| Age: 55 years | Females | | Males | |
| t0            | 83.61   | [79.13 - 88.1]  | 86.54 | [81.94 - 91.14] |
| t1            | 85.16   | [80.82 - 89.49] | 89.03 | [84.61 - 93.46] |
| t3            | 86.78   | [82.34 - 91.23] | 90.03 | [85.48 - 94.58] |
| t6            | 86.95   | [82.44 - 91.46] | 86.59 | [81.96 - 91.23] |
| t12           | 87.17   | [82.66 - 91.69] | 87.43 | [82.79 - 92.07] |

| Tiffeneau percentage** | Females | | Males | |
|-------------------------|---------|---|---|---|
| Age: 75 years | Females | | Males | |
| t0            | 75.25   | [67.39 - 83.1]  | 92.6  | [83.37 - 101.83] |
| t1            | 77.18   | [69.66 - 84.71] | 93.48 | [84.6 - 102.37] |
| t3            | 78.89   | [71.13 - 86.66] | 91.62 | [82.48 - 100.76] |
| t6            | 78.4    | [70.49 - 86.32] | 85.65 | [76.35 - 94.95] |
| t12           | 79.22   | [71.29 - 87.15] | 88.66 | [79.35 - 97.97] |

| ACT score*** | Females | | Males | |
|--------------|---------|---|---|---|
| Age: 25 years | Females | | Males | |
| t0            | 14.37   | [10.67 - 18.07] | 17.05 | [13.06 - 21.04] |
| t1            | 20.21   | [17.93 - 22.49] | 21.24 | [18.83 - 23.66] |
| t3            | 22.81   | [20.67 - 24.95] | 22.46 | [20.2 - 24.71]  |
| t6            | 21.82   | [20.22 - 21.04] | 23.07 | [21.43 - 24.71] |
| t12           | 22.36   | [20.9 - 23.82]  | 23.77 | [22.3 - 25.25]  |

| ACT score*** | Females | | Males | |
|--------------|---------|---|---|---|
| Age: 55 years | Females | | Males | |
| t0            | 17.83   | [16.1 - 19.55]  | 17.63 | [15.64 - 19.62] |
| t1            | 21.74   | [20.65 - 22.83] | 21.66 | [20.43 - 22.88] |
| t3            | 22.56   | [21.53 - 23.6]  | 23.44 | [22.29 - 24.6]  |
| t6            | 23.35   | [22.55 - 24.15] | 23.41 | [22.54 - 24.27] |
| t12           | 23.45   | [22.7 - 24.19]  | 23.9  | [23.11 - 24.7]  |

| ACT score*** | Females | | Males | |
|--------------|---------|---|---|---|
| Age: 75 years | Females | | Males | |
| t0            | 20.13   | [16.9 - 23.36]  | 18.01 | [14.23 - 21.8]  |
| t1            | 22.76   | [20.81 - 24.72] | 21.93 | [19.58 - 24.28] |
| t3            | 22.4    | [20.57 - 24.23] | 24.1  | [21.9 - 26.31]  |
| t6            | 24.37   | [23.04 - 25.69] | 23.63 | [21.97 - 25.3]  |
| t12           | 24.17   | [22.97 - 25.37] | 23.99 | [22.46 - 25.52] |
*Adjusted for: N comorbidity = 2.65, BMI = 24.5, exhaled NO = 61.7, Blood eosinophilia = 642.7; **Adjusted for: N comorbidity = 2.65, BMI = 24.5, exhaled NO = 61.6, Blood eosinophilia = 654.7; ***Adjusted for: N comorbidity = 2.63, BMI = 24.8, exhaled NO = 63.8, Blood eosinophilia = 654.8

Figure S3 – Predicted values (coloured lines) and confidence intervals (shaded contours) for FEV1% of females (pink) and males (light blue) at the age of 25, 55, 75 years old. All the other variables in the model were held constant at their mean value (for numeric vectors) or at their factor's category proportions (for factor variables).
Table S5 – Estimated parameters of LMMs with natural cubic spline interaction between time, sex, and age (adjusted for: biological-drug, history of smoke, BMI, number of comorbidities, exhaled NO, and blood eosinophilia) for FEV1%, Tiffeneau% and ACT score.

### FEV1 percentage*

| Parameter | Value | Std.Error | DF  | t-value | p-value |
|-----------|-------|-----------|-----|---------|---------|
| (Intercept) | 78.05 | 20.31 | 207.00 | 3.84 | <0.001 |
| ns(time, knots = c(1, 6))1 | -7.97 | 14.63 | 207.00 | -0.55 | 0.586 |
| ns(time, knots = c(1, 6))2 | 10.30 | 16.97 | 207.00 | 0.61 | 0.545 |
| ns(time, knots = c(1, 6))3 | -1.98 | 9.62 | 207.00 | -0.21 | 0.837 |
| SESSOM | -41.74 | 18.29 | 45.00 | -2.28 | 0.027 |
| age | -0.43 | 0.24 | 45.00 | 1.78 | 0.081 |
| Biological-drug (Omalizumab) | 8.86 | 5.03 | 45.00 | 1.76 | 0.085 |
| History of smoke (yes) | -3.01 | 5.20 | 45.00 | -0.58 | 0.566 |
| N° comorbidity | 2.60 | 2.06 | 45.00 | 1.26 | 0.214 |
| BMI | -0.01 | 0.63 | 45.00 | -0.22 | 0.805 |
| Exhaled NO baseline | 0.07 | 0.04 | 45.00 | 1.68 | 0.100 |
| Blood eosinophilia baseline | 0.00 | 0.00 | 45.00 | 0.32 | 0.749 |
| ns(time, knots = c(1, 6))1:Sex(M) | 25.23 | 21.14 | 207.00 | 1.19 | 0.234 |
| ns(time, knots = c(1, 6))2:Sex(M) | 43.47 | 24.63 | 207.00 | 1.77 | 0.079 |
| ns(time, knots = c(1, 6))3:Sex(M) | 9.90 | 13.95 | 207.00 | 0.71 | 0.479 |
| ns(time, knots = c(1, 6))1:age | 0.29 | 0.27 | 207.00 | 1.05 | 0.297 |
| ns(time, knots = c(1, 6))2:age | 0.16 | 0.32 | 207.00 | 0.51 | 0.609 |
| ns(time, knots = c(1, 6))3:age | 0.08 | 0.18 | 207.00 | 0.43 | 0.699 |
| SESSOM:age | 0.87 | 0.35 | 45.00 | 2.50 | 0.016 |
| ns(time, knots = c(1, 6))1:Sex(M):age | -0.66 | 0.40 | 207.00 | -1.65 | 0.100 |
| ns(time, knots = c(1, 6))2:Sex(M):age | -1.09 | 0.47 | 207.00 | -2.33 | 0.021 |
| ns(time, knots = c(1, 6))3:Sex(M):age | -0.19 | 0.26 | 207.00 | -0.71 | 0.478 |

### Tiffeneau percentage**

| Parameter | Value | Std.Error | DF  | t-value | p-value |
|-----------|-------|-----------|-----|---------|---------|
| (Intercept) | 100.48 | 12.33 | 208.00 | 8.15 | <0.001 |
| ns(time, knots = c(1, 6))1 | 5.19 | 7.99 | 208.00 | 0.65 | 0.517 |
| ns(time, knots = c(1, 6))2 | 4.28 | 8.71 | 208.00 | 0.49 | 0.624 |
| ns(time, knots = c(1, 6))3 | 1.90 | 4.84 | 208.00 | 0.39 | 0.695 |
| Sex(M) | -36.75 | 10.91 | 45.00 | -3.37 | 0.002 |
| age | -0.42 | 0.14 | 45.00 | -2.90 | 0.006 |
| Biological-drug (Omalizumab) | 4.40 | 3.09 | 45.00 | 1.43 | 0.161 |
| History of smoke (yes) | -4.44 | 3.20 | 45.00 | -1.39 | 0.172 |
| N° comorbidity | 2.64 | 1.27 | 45.00 | 2.09 | 0.043 |
| BMI | -0.07 | 0.39 | 45.00 | -0.18 | 0.857 |
| Exhaled NO baseline | 0.01 | 0.02 | 45.00 | 0.52 | 0.609 |
| Blood eosinophilia baseline | 0.00 | 0.00 | 45.00 | 0.08 | 0.940 |
| ns(time, knots = c(1, 6))1:Sex(M) | 11.50 | 11.57 | 208.00 | 0.99 | 0.322 |
| ns(time, knots = c(1, 6))2:Sex(M) | 26.27 | 12.62 | 208.00 | 2.08 | 0.038 |
| ns(time, knots = c(1, 6))3:Sex(M) | 3.34 | 7.00 | 208.00 | 0.48 | 0.634 |
| ns(time, knots = c(1, 6))1:age | -0.06 | 0.15 | 208.00 | -0.39 | 0.695 |
| ns(time, knots = c(1, 6))2:age | 0.04 | 0.16 | 208.00 | 0.25 | 0.805 |
| ns(time, knots = c(1, 6))3:age | -0.01 | 0.09 | 208.00 | -0.07 | 0.943 |
| Sex(M):age | 0.72 | 0.21 | 45.00 | 3.45 | 0.001 |
| ns(time, knots = c(1, 6))1:Sex(M):age | -0.34 | 0.22 | 208.00 | -1.53 | 0.128 |
| ns(time, knots = c(1, 6))2:Sex(M):age | -0.51 | 0.24 | 208.00 | -2.13 | 0.034 |
| ns(time, knots = c(1, 6))3:Sex(M):age | -0.14 | 0.13 | 208.00 | -1.02 | 0.311 |

### ACT score***

| Parameter | Value | Std.Error | DF  | t-value | p-value |
|-----------|-------|-----------|-----|---------|---------|
| (Intercept) | 11.98 | 3.61 | 180.00 | 3.32 | 0.001 |
| ns(time, knots = c(1, 3, 6))1 | 12.29 | 3.34 | 180.00 | 3.68 | <0.001 |
| ns(time, knots = c(1, 3, 6))2 | 3.20 | 2.68 | 180.00 | 1.19 | 0.235 |
| ns(time, knots = c(1, 3, 6))3 | 18.00 | 6.80 | 180.00 | 2.65 | 0.009 |
| ns(time, knots = c(1, 3, 6))4 | 4.43 | 1.68 | 180.00 | 2.63 | 0.009 |
| Sex(M) | 0.08 | 4.76 | 39.00 | 0.07 | 0.929 |
| age | 0.12 | 0.06 | 39.00 | 1.93 | 0.061 |
| Biological-drug (Omalizumab) | 0.53 | 0.53 | 39.00 | 1.00 | 0.325 |
| History of smoke (yes) | -0.23 | 0.60 | 39.00 | -0.38 | 0.704 |
| N° comorbidities | -0.12 | 0.21 | 39.00 | -0.55 | 0.585 |
| BMI | -0.01 | 0.07 | 39.00 | -0.09 | 0.930 |
|                          |       |       |       |       |       |
|--------------------------|-------|-------|-------|-------|-------|
| Exhaled NO baseline      | 0.00  | 0.00  | 39.00 | -0.59 | 0.561 |
| Blood eosinophilia baseline | 0.00  | 0.00  | 39.00 | -0.18 | 0.858 |
| ns(time, knots = c(1, 3, 6))1:Sex(M) | -8.31 | 4.95  | 180.00 | -1.68 | 0.095 |
| ns(time, knots = c(1, 3, 6))2:Sex(M) | 2.10  | 3.98  | 180.00 | 0.53  | 0.598 |
| ns(time, knots = c(1, 3, 6))3:Sex(M) | -5.09 | 10.09 | 180.00 | -0.51 | 0.614 |
| ns(time, knots = c(1, 3, 6))4:Sex(M) | -0.70 | 2.50  | 180.00 | -0.28 | 0.778 |
| ns(time, knots = c(1, 3, 6))1:age | -0.15 | 0.06  | 180.00 | -2.46 | 0.015 |
| ns(time, knots = c(1, 3, 6))2:age | 0.02  | 0.05  | 180.00 | 0.48  | 0.630 |
| ns(time, knots = c(1, 3, 6))3:age | -0.13 | 0.13  | 180.00 | -1.00 | 0.320 |
| ns(time, knots = c(1, 3, 6))4:age | -0.03 | 0.03  | 180.00 | -1.07 | 0.286 |
| Sex(M):age               | -0.10 | 0.09  | 39.00  | -1.07 | 0.289 |
| ns(time, knots = c(1, 3, 6))1:Sex(M):age | 0.18  | 0.09  | 180.00 | 1.98  | 0.049 |
| ns(time, knots = c(1, 3, 6))2:Sex(M):age | -0.05 | 0.08  | 180.00 | -0.70 | 0.483 |
| ns(time, knots = c(1, 3, 6))3:Sex(M):age | 0.09  | 0.19  | 180.00 | 0.50  | 0.618 |
| ns(time, knots = c(1, 3, 6))4:Sex(M):age | 0.03  | 0.05  | 180.00 | 0.53  | 0.600 |

*Number of Observations: 274, Number of Groups: 55; **Number of Observations: 275, Number of Groups: 55; ***Number of Observations: 245, Number of Groups: 49
