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

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ABSTRACT

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 p atients 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/m^2. 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 relevantdifference existed in general between sexesat 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 0.85mg-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 (19-47years: mean 0.86 mg-median 1.00 mg [1.00-1.00]; 48-56years: mean 0.82 mg-median 1.00 mg [0.90-1.00]; 57-83years: 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 12^{th} 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

*R*aw 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.

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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)	Female			Male			**Total females (n=37)**	**Total males (n=27)**
		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)		
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	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 (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%)	6 (60%)	8 (80%)	9 (100%)	5 (83%)	9 (82%)	27 (82%)	23 (89%)
Autoimmune diseases (n=58-N/A=6)									
no	55 (95%)	12 (92%)	9 (90%)	9 (90%)	9 (100%)	6 (100%)	10 (100%)	30 (91%)	25 (100%)
yes	3 (5%)	1 (8%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	3 (9%)	0 (0%)
Interstitial lung disease (58-N/A=6)									
no	56 (97%)	12 (92%)	9 (90%)	10 (100%)	9 (100%)	6 (100%)	10 (100%)	31 (94%)	25 (100%)
yes	2 (3%)	1 (8%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (6%)	0 (0%)
Dermatitis (58-N/A=6)									
no	56 (97%)	12 (92%)	10 (100%)	9 (90%)	9 (100%)	6 (100%)	10 (100%)	31 (94%)	25 (100%)
yes	2 (3%)	1 (8%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	2 (6%)	0 (0%)
Gastro-Esophageal Reflux Disease (GERD) **(58-N/A=6)**									
no	47 (81%)	10 (77%)	9 (90%)	5 (50%)	8 (89%)	6 (100%)	9 (90%)	24 (73%)	23 (92%)
yes	11 (19%)	3 (23%)	1 (10%)	5 (50%)	1 (11%)	0 (0%)	1 10.0 (0%)	9 (27%)	2 (8%)
Biologic drugs (n=61-N/A=3)									
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%)	6 (60%)	7 (78%)	4 (67%)	5 (46%)	19 (54%)	16 (62%)
Oral corticosteroids (mg) (n64-N/A=0)									
Median	2.5	5,0	0,0	2.5	0,0	2.5	5,0	0,0	5,0
(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 (cells/mm3) (n=61-N/A=3)									
Median	570,0	720,0	880,0	340,0	500,0	420,0	400,0	650,0	435,0

(25th-75th centiles)	(300.0-860.0)	(600.0-960.0)	(345.0-1080.0)	(272.5-552.5)	(430.0-680.0)	(282.5-760.0)	(272.5-707.5)	(320.0-1000.0)	(275.0-702.5)
Exhaled NO (ppb) (n=61-N/A=3)									
Median	48.2	37.12	74,0	39.66	41.4	59,0	55.4	49,0	45,0
(25th-75th centiles)	(28.2-87.5)	(27.4-92.2)	(57.0-99.8)	(20.3-48.0)	(34.7-45.0)	(30.3-83.9)	(29.6-89.3)	(27.1-93.0)	(30.9-79.7)
Fev1 % t0 (n=63-N/A=1)									
Median	70,0	85,0	64.5	64,0	69,0	76,0	77,0	68,0	73.5
(25th-75th centiles)	(59.5-83.5)	(68.0-93.0)	(44.5-76.8)	(59.8-81.5)	(62.0-76.0)	(66.0-81.8)	(66.0-80.0)	(56.0-87.0)	(62.8-80.5)
FVC % t0 (n=63-N/A=1)									
Median	86,0	88,0	75.5	88,0	83,0	92,0	85,0	88,0	84,0
(25th-75th centiles)	(73.0-98.5)	(69.0-103.0)	(63.3-94.5)	(87.3-96.3)	(65.0-92.0)	(79.3-109.3)	(80.5-88.0)	(69.0-103.0)	(77.5-92.8)
Tiffeanu % t0 (n=62-N/A=2)									
Median	87.5	97,0	82,0	78.5	83,0	83,0	91,0	87.5	87,0
(25th-75th centiles)	(77.3-97.0)	(88.0-101.0)	(73.0-89.0)	(75.5-90.0)	(81.0-91.0)	(74.3-88.0)	(84.0-97.5)	(77.0-98.2)	(80.2-94.5)
ACT t0 (n=62-N/A=2)									
Median	17,0	15,0	17,0	18.5	18,0	16,0	18,0	17,0	18,0
(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			**Males**		
	Value	Std. Error	p-value	Value	Std. Error	p-value
	FEV 1 percentage (n=37; n° obs=179)			**FEV 1 percentage (n=27; n° obs=129)**		
(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 (n=36; n° obs=177)			**Tiffeneau percentage (n=27; n° obs=130)**		
(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 (n=34; n° obs=158)			**ACT score (n=22; n° obs=106)**		
(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.

	Females		Males	
	Predicted	95% CI	Predicted	95% CI
FEV 1 percentage*				
Age: 35 years				
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]
Age: 50 years				
t0	70.95	[63.46 -78.43]	72.92	[65.73 -80.11]
t1	75.75	[68.55 -82.95]	74.96	[68.11 -81.80]
t3	80.85	[73.42 -88.28]	76.31	[69.16 -83.46]
t6	81.11	[73.59 -88.63]	75.04	[67.80 -82.27]
t12	79.11	[71.58 -86.63]	78.04	[70.80 -85.29]
Age: 70 years				
t0	62.33	[51.13 -73.53]	81.79	[68.74 -94.83]
t1	67.29	[56.64 -77.94]	79.19	[66.73 -91.64]
t3	73.65	[62.56 -84.74]	75.36	[62.43 -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]
Tiffeneau percentage**				
Age: 35 years				
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 -87.00]	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]
ACT score***				
Age: 35 years				
t0	15.52	[12.84 -18.20]	17.24	[14.37 -20.12]
t1	20.72	[19.05 -22.39]	21.38	[19.65 -23.12]
t3	22.73	[21.15 -24.30]	22.79	[21.16 -24.41]
t6	22.33	[21.13 -20.12]	23.18	[22.01 -24.35]
t12	22.72	[21.62 -23.83]	23.82	[22.76 -24.87]
Age: 50 years				
t0	17.25	[15.54 -18.96]	17.53	[15.62 -19.44]
t1	21.49	[20.39 -22.58]	21.59	[20.42 -22.75]
t3	22.6	[21.57 -23.64]	23.28	[22.18 -24.37]
t6	23.09	[22.28 -23.91]	23.35	[22.54 -24.16]
t12	23.27	[22.51 -24.02]	23.88	[23.15 -24.62]
Age: 70 years				
t0	19.56	[16.82 -22.29]	17.92	[14.7 -21.13]
t1	22.51	[20.84 -24.17]	21.86	[19.86 -23.86]
t3	22.44	[20.88 -24.00]	23.94	[22.06 -25.82]
t6	24.11	[22.97 -25.25]	23.58	[22.16 -25.00]
t12	23.99	[22.95 -25.02]	23.97	[22.66 -25.28]

***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

Figure 1

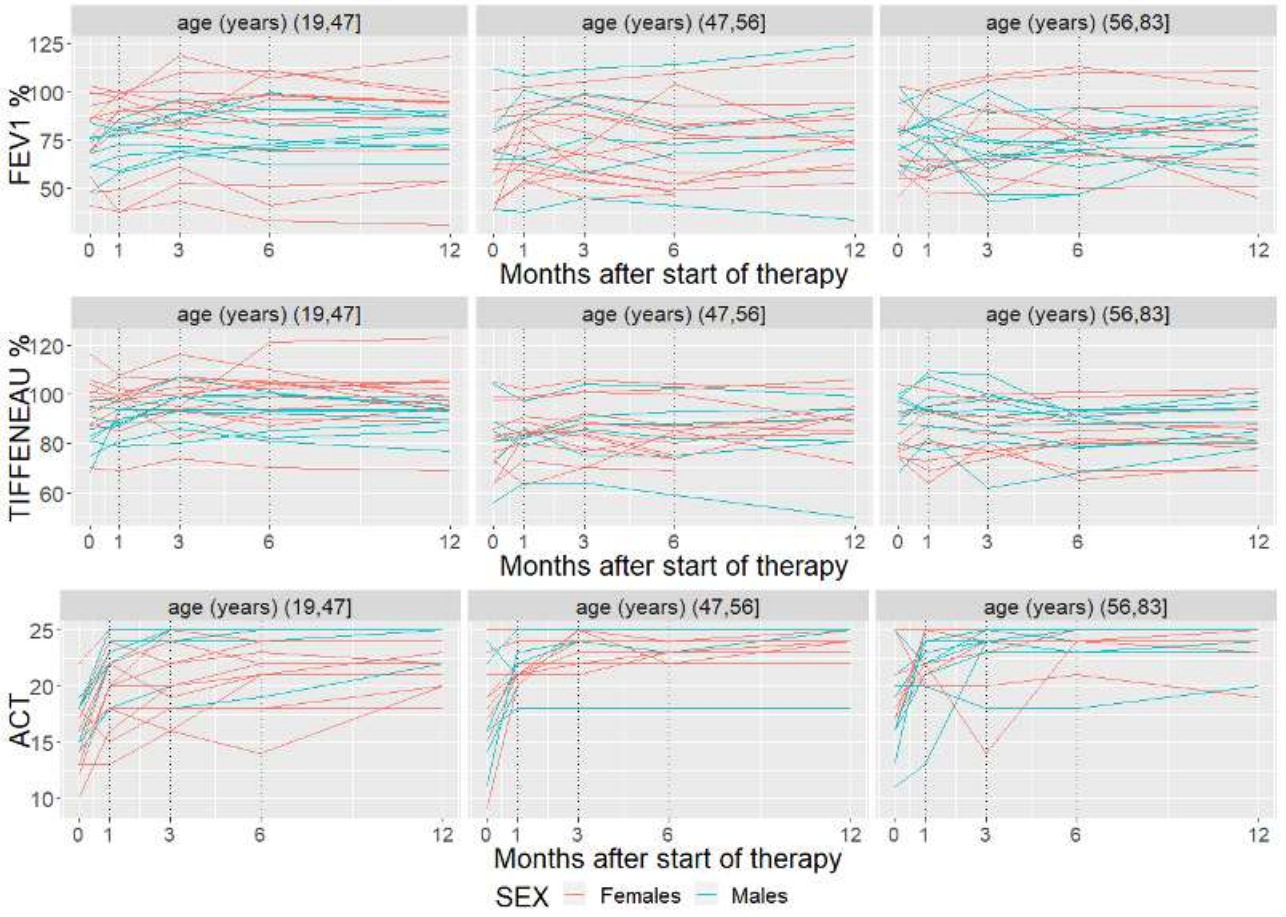

age (years) (19,47]
age (years) (47,56]
age (years) (56,83]
FEV1 %
125
100
75
50
0 1 3 6 12
Months after start of therapy
TIFFENEAU %
120
100
80
60
ACT
25
20
15
10
SEX
Females
Males

Figure 2

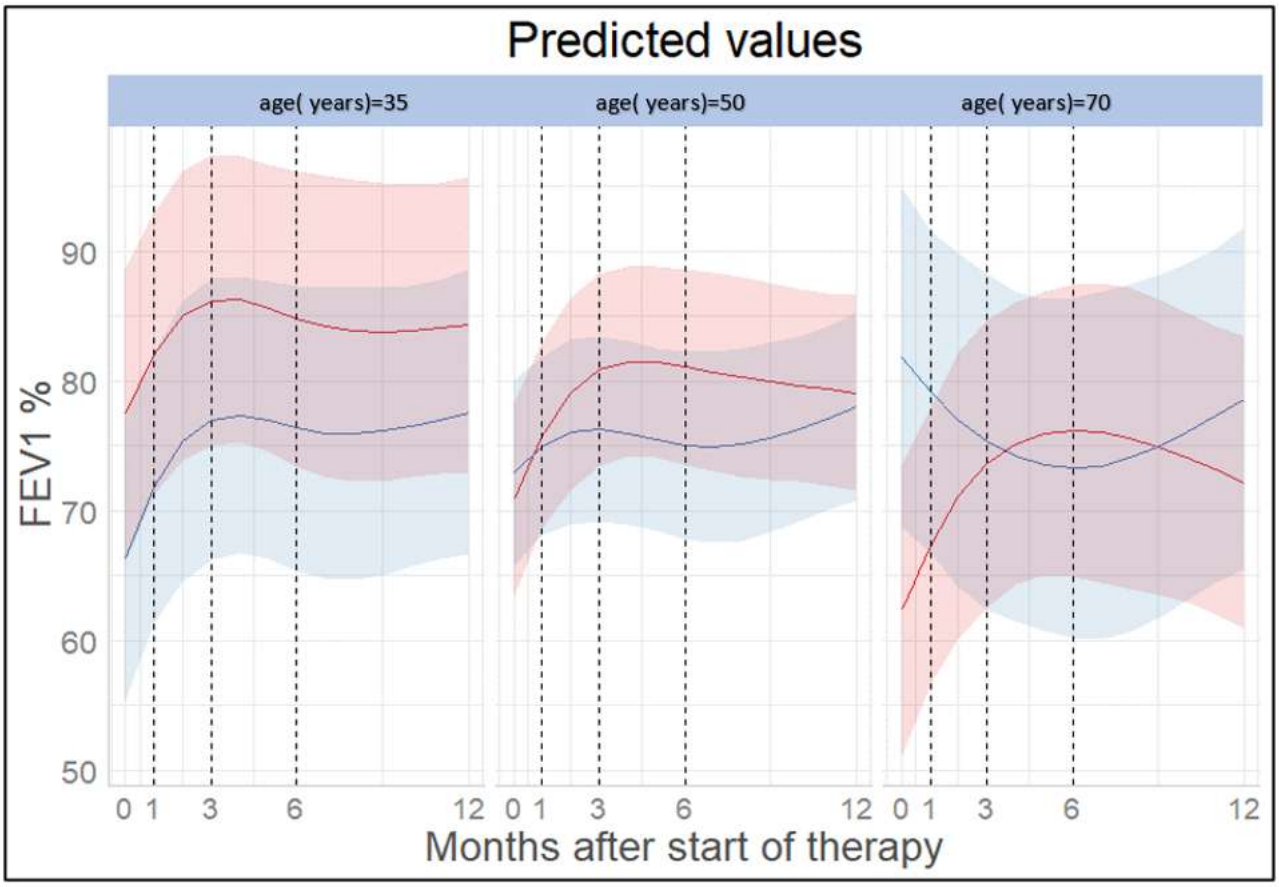
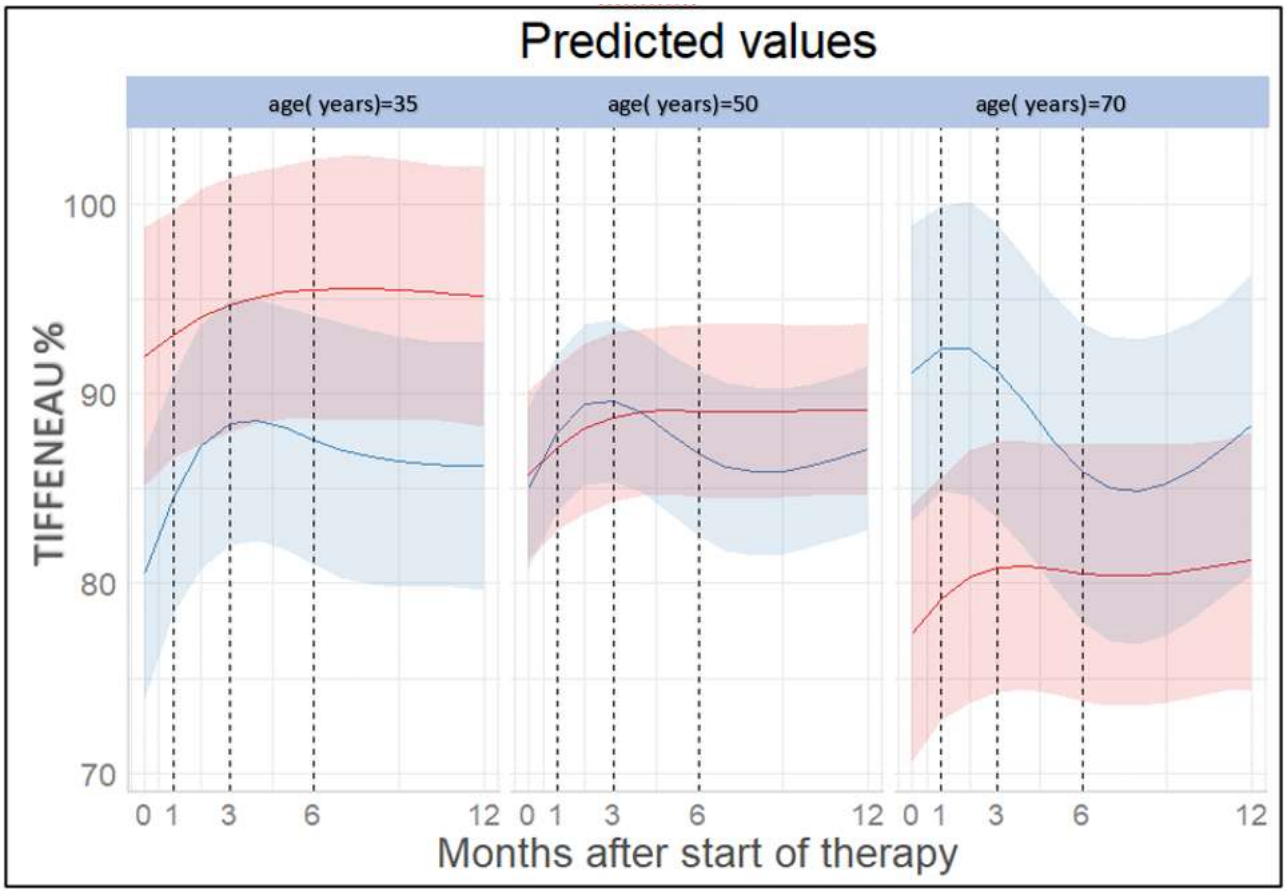

Predicted values
age(years)=35
age(years)=50
age(years)=70
FEV1 %
90
80
70
60
50
0 1 3 6 12
0 1 3 6 12
0 1 3 6 12
Months after start of therapy

Figure 3


Predicted values
age(years)=35
age(years)=50
age(years)=70
TIFFENEAU %
100
90
80
70
0 1 3 6 12
Months after start of therapy

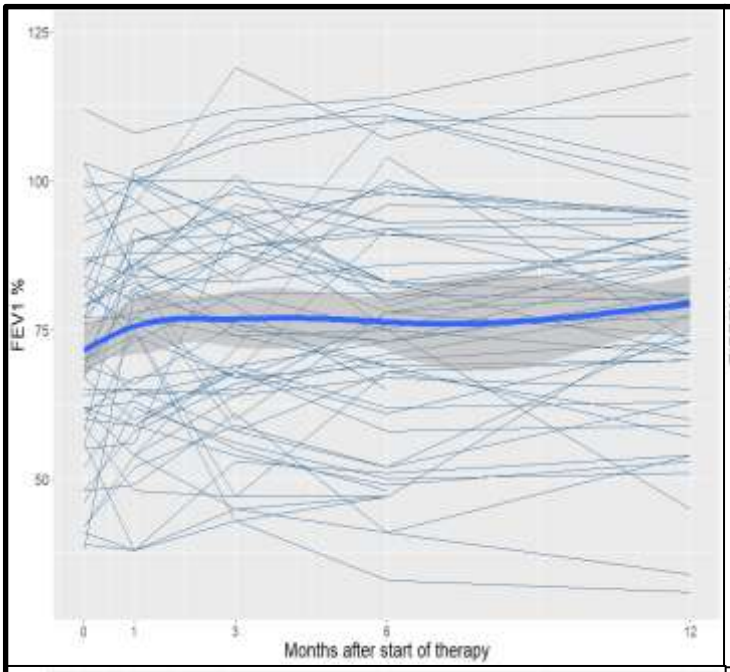
SUPPLEMENTARY FILE

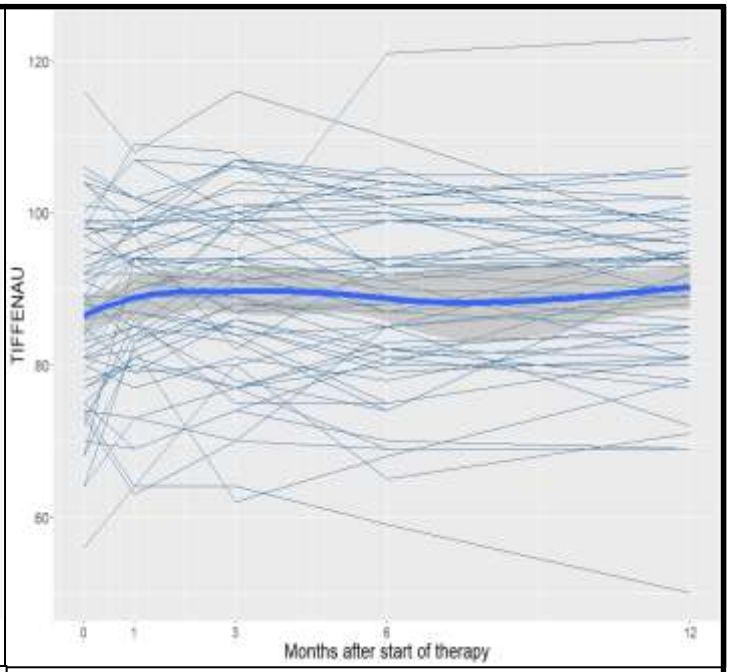
Table S1 - Baseline characteristics of patient with severe asthma distinguished by age group (tertiles). Percentage and median with interquartile range (IQR) were used.

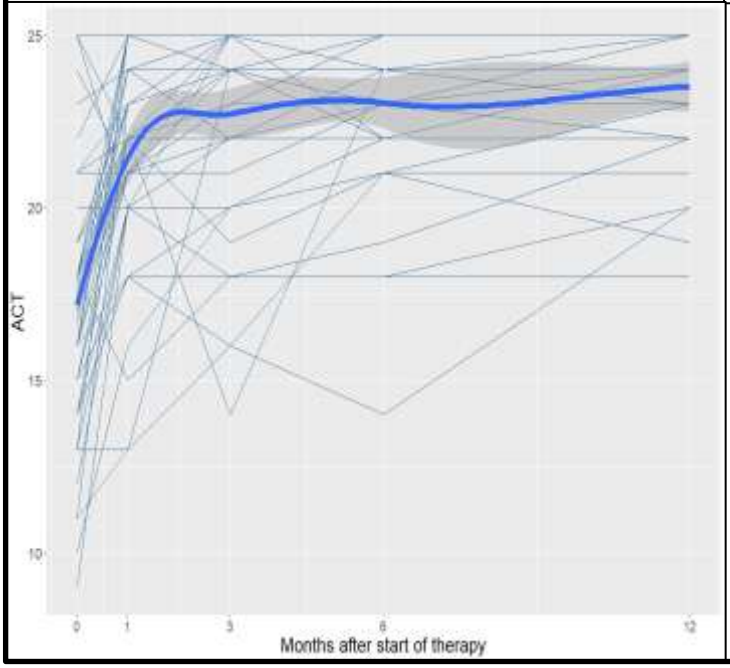
	N° (64)	Age group 19-47 years (n=22)	48-56 years (n=20)	57-83 years (n=22)
BMI (Kg/m^2)	62			
Median (IQR)		22.6 (20.0, 27.2)	23.3 (21.5, 25.5)	26.2 (24.2, 27.7)
History of smoke	58			
no		17 (77.3)	13 (81.2)	16 (80.0)
yes		5 (22.7)	3 (18.8)	4 (20.0)
N° comorbidity	64			
Median (IQR)		3.00 (2.00, 3.00)	2.50 (1.75, 3.00)	2.00 (2.00, 3.00)
Polyposis	62			
no		10 (45.5)	6 (31.6)	12 (57.1)
yes		12 (54.5)	13 (68.4)	9 (42.9)
Bronchiectasis	57			
no		19 (90.5)	14 (87.5)	20 (100.0)
yes		2 (9.5)	2 (12.5)	0 (0.0)
Rhinitis	58			
no		0 (0.0)	1 (6.2)	3 (15.0)
yes		22 (100.0)	15 (93.8)	17 (85.0)
Atopy	59			
no		0 (0.0)	5 (31.2)	4 (19.0)
yes		22 (100.0)	11 (68.8)	17 (81.0)
Autoimmune diseases	58			
no		21 (95.5)	15 (93.8)	19 (95.0)
yes		1 (4.5)	1 (6.2)	1 (5.0)
Interstitial lung disease	58			
no		21 (95.5)	15 (93.8)	20 (100.0)
yes		1 (4.5)	1 (6.2)	0 (0.0)
Dermatitis	58			
no		21 (95.5)	16 (100.0)	19 (95.0)
yes		1 (4.5)	0 (0.0)	1 (5.0)
MRGE	58			
no		18 (81.8)	15 (93.8)	14 (70.0)
yes		4 (18.2)	1 (6.2)	6 (30.0)
Biologic-drug therapy	61			
Mepolizumab		4 (18.2)	12 (66.7)	10 (47.6)
Omalizumab		18 (81.8)	6 (33.3)	11 (52.4)
OCS (mg)	64			
Median (IQR)		0.00 (0.00, 5.00)	0.00 (0.00, 5.00)	5.00 (0.00, 11.88)
Exhaled NO (ppb)	61			
Median (IQR)		38.4 (29.6, 65.5)	73.0 (47.0, 97.6)	46.5 (21.9, 86.6)
Blood eosinophilia (cells/mm^3)	62			
Median (IQR)		645.0 (432.5, 862.5)	740.0 (287.5, 1005.0)	400.0 (260.0, 702.5)
FEV1 percentage	63			
Median (IQR)		75.0 (63.5, 85.8)	66.0 (49.5, 81.2)	73.0 (62.0, 81.0)
FVC percentage	63			
Median (IQR)		85.5 (68.2, 101.0)	80.5 (68.5, 100.2)	87.0 (83.0, 91.0)
Tiffeneau percentage	62			
Median (IQR)		91.5 (83.8, 98.8)	82.0 (73.0, 89.0)	88.0 (77.0, 93.0)
ACT score	62			
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.







	Overall		Female		Male	
	Mean	SD	Mean	SD	Mean	SD
Fev 1 %						
t0	71.6	18.29	70.65	20,06	73.00	15.71
t1	75.6	17.65	75.00	19,57	76.46	14.93
t3	76.8	19.15	77.67	20,18	75.6	17.91
t6	76.3	20.12	77.42	22,63	74.81	16.34
t12	79.5	19.04	79.44	21,08	79.46	16.40
Tiffeneau %						
t0	86.5	12.43	70.65	20,06	73.00	15.71
t1	88.8	10.96	75.00	19,57	76.46	14.93
t3	89.7	12.21	77.67	20,18	75.6	17.91
t6	88.7	12.49	77.42	22,63	74.81	16.34
t12	90.3	11.46	79.44	21,08	79.46	16.40
ACT score						
t0	17.2	4.00	17,06	4,37	17,38	3,51
t1	21.4	2.82	21,24	2,91	21,72	2,75
t3	22.7	2.80	22,18	3,07	23,4	2,29
t6	23.0	2.29	22,76	2,41	23,4	2,10
t12	23.5	1.84	23,18	1,86	23,92	1,75

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).

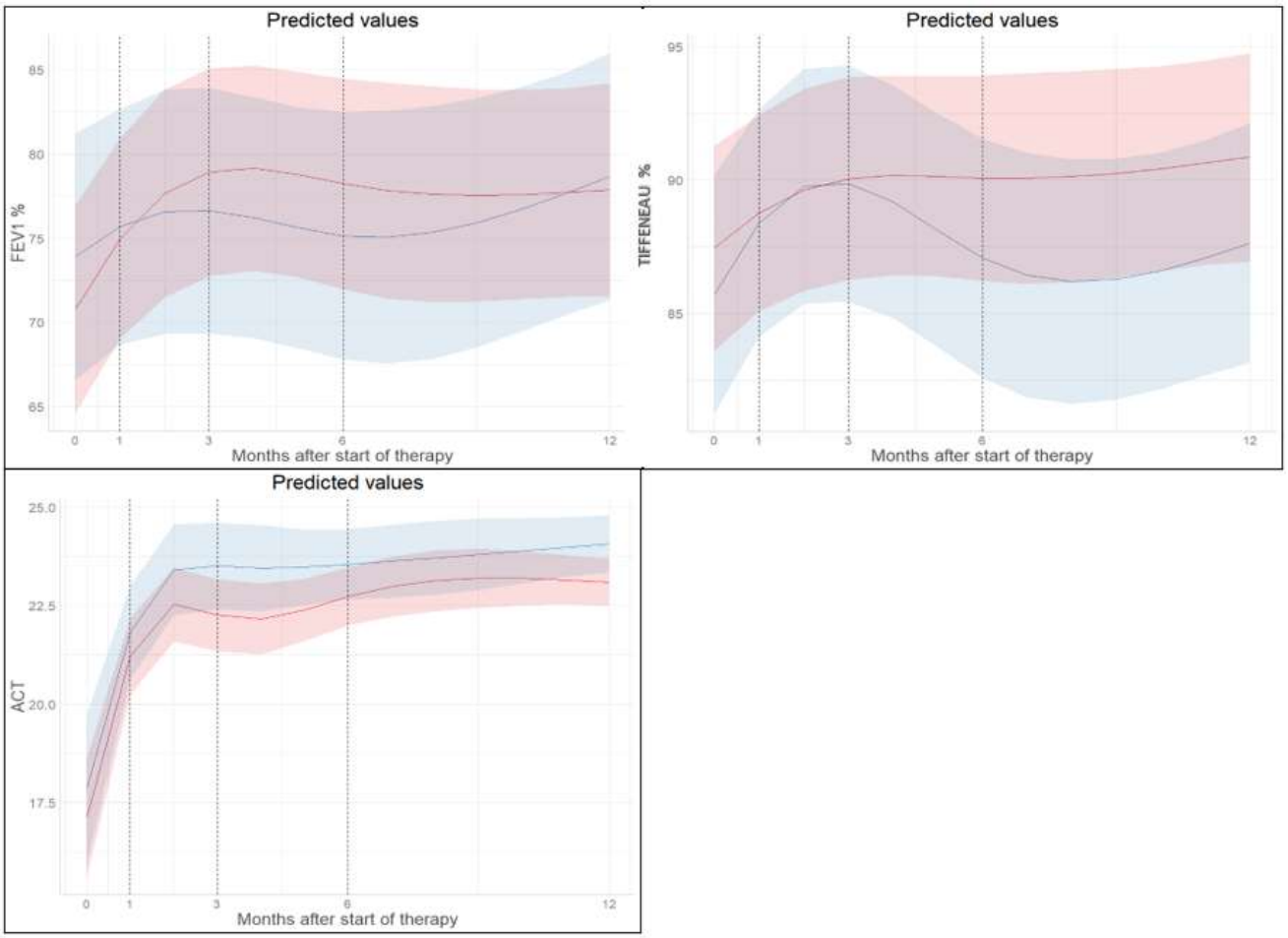


Table S2 – Estimated parameters of LMMs with natural cubic spline with interaction between time and sex for FEV1%, Tiffeneau% and ACT score.

Fev1 percentage					
	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	0.65	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					
	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					
	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

FEV 1 percentage*					
	Value	**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**					
	Value	**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***					
	Value	**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.

	Females		Males	
	Predicted	**95% CI**	**Predicted**	**95% CI**
		FEV 1 percentage*		
Age: 25 years				
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				
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				
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**		
Age: 25 years				
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]
Age: 55 years				
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]
Age: 75 years				
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***		
Age: 25 years				
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]
Age: 55 years				
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]
Age: 75 years				
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).

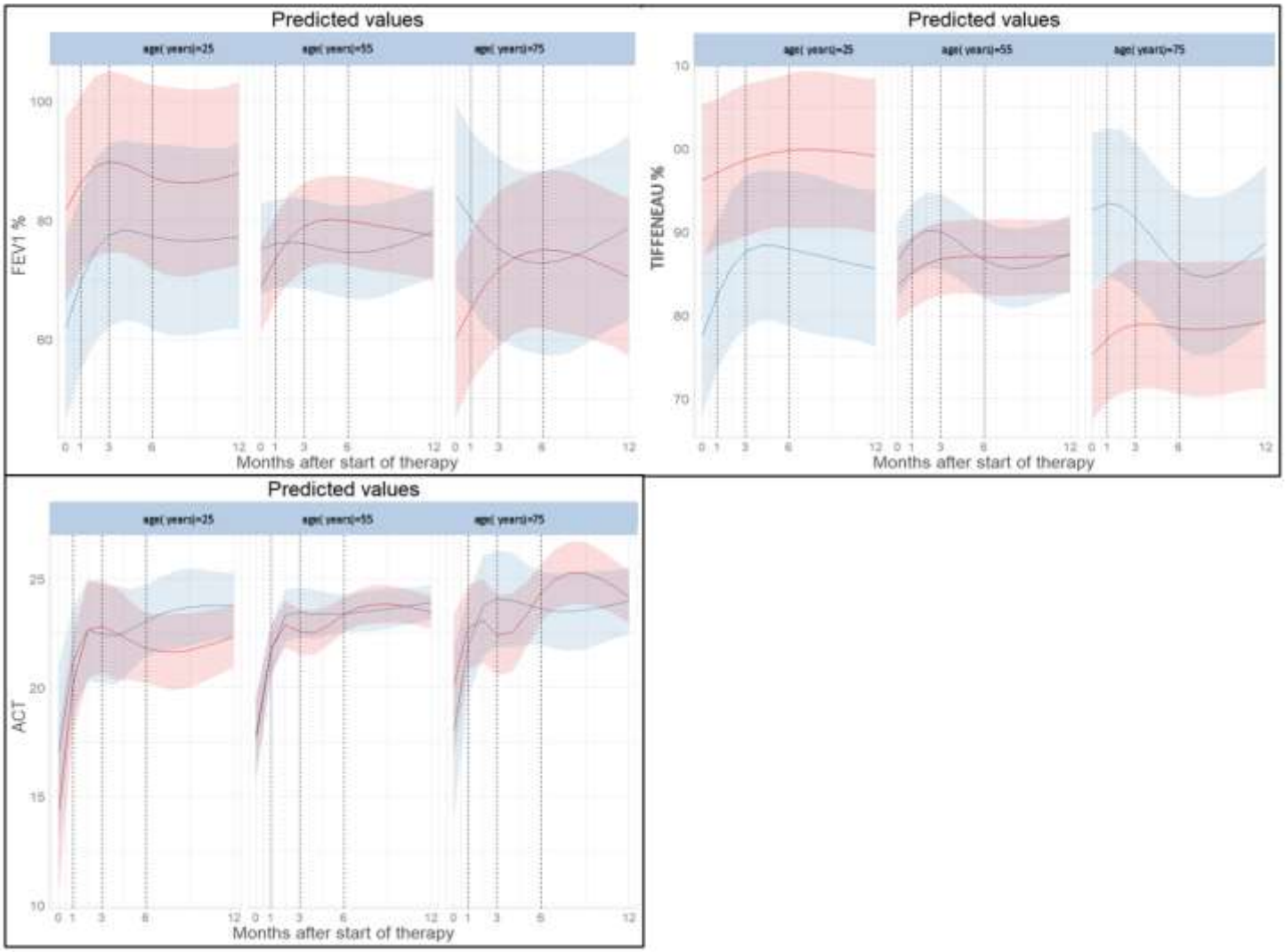


Table S5 – Estimated parameters of LMMs with natural cubic spline with interaction between time, sex, and age (adjusted for: biological-drug, history of smoke, BMI, number of comorbidities, exhaled NO, and blood eosinophilia) for FEV1%, Tiffeneau% and ACT score.

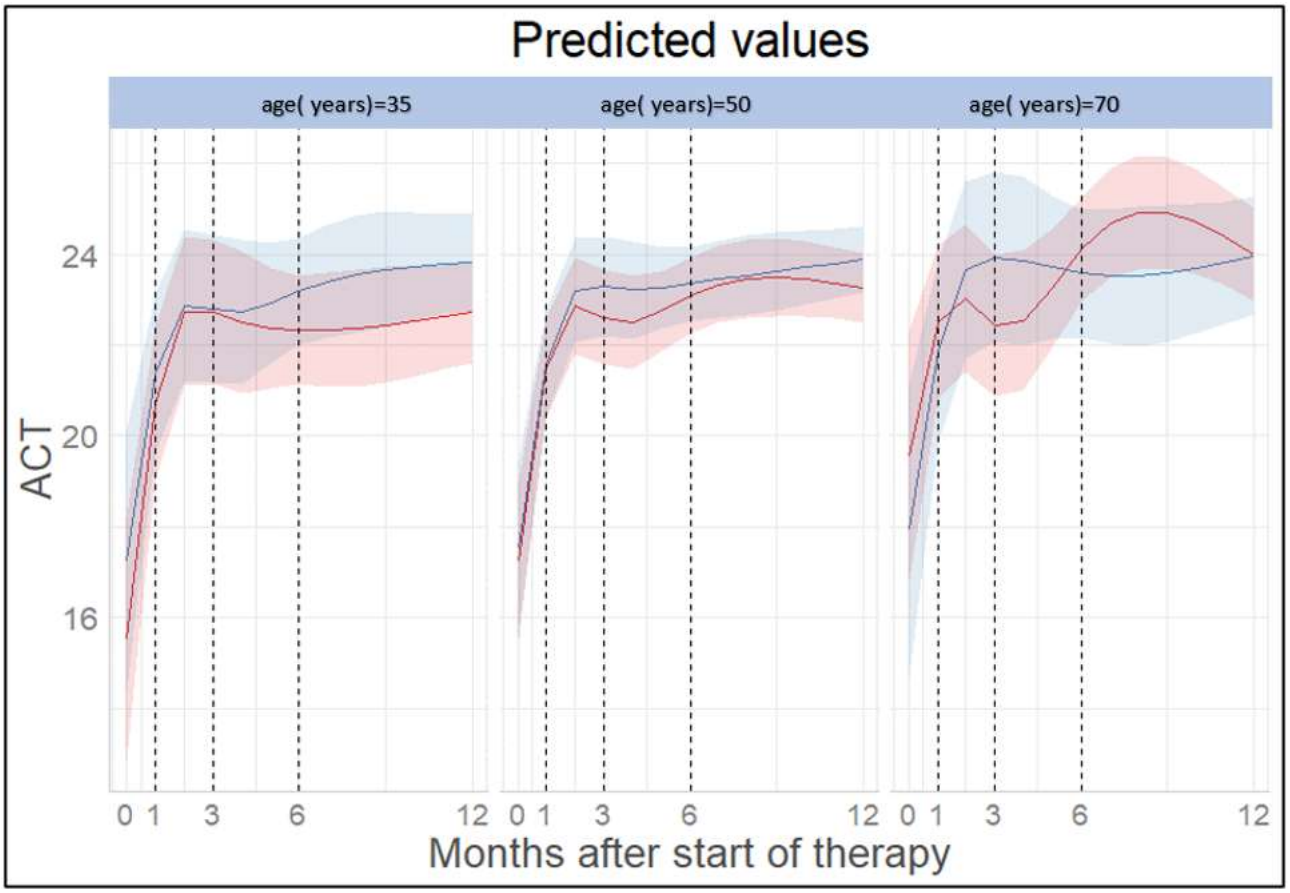
FEV 1 percentage*					
	Value	**Std.Error**	**DF**	**t-value**	**p-value**
(Intercept)	78.05	20.31	207.00	3.84	<0.001
ns(time, knots = c(1, 6))1	-7.97	14.63	207.00	-0.55	0.586
ns(time, knots = c(1, 6))2	10.30	16.97	207.00	0.61	0.545
ns(time, knots = c(1, 6))3	-1.98	9.62	207.00	-0.21	0.837
SESSOM	-41.74	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	-0.02	0.985
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	0.669
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**					
	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***					
	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)	5.08	4.76	39.00	1.07	0.292
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

Figure 4


Predicted values
age(years)=35
age(years)=50
age(years)=70
ACT
24
20
16
0 1 3 6 12
0 1 3 6 12
0 1 3 6 12
Months after start of therapy