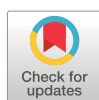# Clinical response to biologicals for severe asthma: any relevance for sex in different age ranges?

Roberto Benoni[1], Silvia Panunzi[2], Veronica Batani [3], Francesca Moretti[1], Stefano Fuggini[1], Mattia Todesco[4], Gianenrico Senna[3,4], Albino Poli[1], Andrea Vianello[5] and Marco Caminati[3]

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

Corresponding author: Veronica Batani (ve.batani@gmail.com)

Abstract

Background Whether sex can influence the clinical response to biological treatment in patients with severe asthma has not been fully addressed.
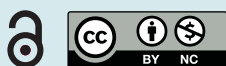
Aims and methods The aim of this study was to investigate in patients with severe asthma undergoing biological treatment the individual evolution of lung function measurements and patient-reported asthma control scores over a 12-month follow-up period, in relation to patients' sex, in different age ranges. Second, the change in the administered dose of oral corticosteroids (OCS) before and after 12 months of treatment was investigated.

Results 64 patients (58% female and 42% male) with a median age of 52 years were enrolled in the study. There were no relevant differences between sexes in terms of lung function, patient-reported asthma control, exacerbation rate and daily OCS dose within the study timeframe. A separate sub-analysis by biological treatment confirmed the same finding. Stratifying individuals by age, we showed that older men had lower lung function parameter values (forced expiratory volume in 1 s (FEV_1) % predicted and FEV_1/ forced vital capacity index) than older women, whereas an opposite trend was observed in terms of Asthma Control Test score. No other relevant differences were detected after age stratification.

Conclusion According to our findings, sex does not act as a determinant of treatment response to biologicals in people with severe asthma. Although to be confirmed in larger studies, our data suggest that neither sex nor age should limit biological treatment prescription, once the eligibility criteria for that therapy are satisfied.

Introduction

Bronchial asthma affects ∼4.5% of the general population, with not negligible differences according to country, age range and sex. In adults, asthma is more prevalent in women, while in younger individuals more boys than girls are reported to be asthmatic. No relevant differences by sex are observed after the menopausal period [1–5]. Overall, <5% of people with asthma have severe disease, according to the European Respiratory Society (ERS)/American Thoracic Society (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 women, and are characterised by more late onset, more comorbidities and poorer asthma control [4]. The complex interplay between hormonal fluctuations, inflammatory mechanisms and environmental factors may account for the sex differences, but this is still a controversial issue. Whether sex can influence the response to biological treatment in patients with severe asthma is also not completely clear. Up to now, evidence on the topic mainly comes from studies

analysing potential predictors of response to biologicals, including sex, which does not seem to have any relevance [9–12]. However, sex has not yet been primarily explored as a factor associated with the clinical response to biological treatment in people with severe asthma.

The aim of the present study was to investigate in patients with severe asthma the individual evolution of lung function measurements and patient-reported asthma control scores over a 12-month follow-up of biological treatment in relation to patients' sex, and in different age ranges. Second, the change in the administered dose of oral corticosteroids (OCS) before and after 12 months of treatment was investigated.

Methods
Study subjects
The study population was sampled from the registry of the Interdisciplinary Network for the management of severe asthma in the Veneto region, Italy, which is 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] and eligibility for treatment with at least one of the biological 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 were excluded. For the present study, all consecutive patients referred to the Verona centre and on biological treatment for at least 12 months at the time of the analysis were considered.

Study design
A retrospective analysis was carried out to explore how sex, overall and in different age ranges, may influence the response to biological therapies in patients with severe asthma. The data used were collected from patients included in the registry who were prescribed biological therapy for severe asthma and regularly followed-up at our centre. Clinical and functional data from visits at 1, 3, 6 and 12 months after the treatment start (t1, t3, t6 and t12, respectively) were collected and analysed.

Data collection
For each subject the following data were included in the analysis: age at the biological treatment start, sex, body mass index (BMI), blood eosinophil counts (expressed as cells·mm^{-3}), exhaled nitric oxide fraction (F_{ENO}) (expressed as parts per billion), smoking history, number of comorbidities, prescribed biological drug, Asthma Control Test (ACT) scores, forced expiratory volume in 1 s (FEV_1) % predicted and Tiffeneau index (FEV_1/forced vital capacity (FVC) %) at baseline and at each visit (t1, t3, t6 and t12). The Tiffeneau index is a validated measure of bronchial obstruction derived from the FEV_1/FVC ratio; it is used to differentiate between obstructive lung disease (in which both FEV_1 and FEV_1/FVC are reduced) and restrictive lung disease (in which FEV_1 is reduced but FEV_1/FVC is normal due to the contemporaneous reduction of both FEV_1 and FVC). Normal Tiffeneau values are >75–80% in adults and >90% in children. Values <80% are suggestive of obstructive respiratory disease, with lower values related to a greater degree of obstruction [16]. The OCS dose at baseline (mg of prednisone or equivalent per day) and post 12 months' treatment as referred by the patient was analysed.

Regarding comorbidities, data were collected as follows: smoking history was considered positive in the case of current or former smokers; polyposis was recorded in the presence of a documented rhinoscopy or facial computed tomography (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 a positive prick test or the detection of serum-specific immunoglobulin E to aero-allergens; and autoimmune diseases, dermatitis and gastro-oesophageal reflux disease (GERD) were determined from the medical records provided by the patients at the time of first assessment at the referral centre for severe asthma.

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] to estimate individual and mean evolutions of FEV_1 % predicted, Tiffeneau % and ACT values for patients over the course of the follow-up and to compare predicted trajectories between men and women of different ages, when adjusting for baseline clinical and demographic characteristics.

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

For the first set of analyses, three random intercept LMMs were fitted for each of the outcomes and for each sex subgroup separately, including measurement time as a fixed factor variable (with levels set up to 1, 3, 6 and 12 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 men and women [18].

In a second set of analyses, the cohort sample was investigated. Natural cubic B-splines [19] were used to parameterise 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 and t12), two inner knots were chosen for FEV_1 % predicted and Tiffeneau % at t1 and t6, and an additional knot was fitted at t3 for ACT outcomes. The Akaike information criterion 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 plausible nonlinear growth to be modelled over months of follow-up, but did not permit direct interpretation of each of the estimated parameters. These models were therefore used to provide age-specific estimates of outcome measures over time, and to graphically depict the association between sex and age and FEV_1 % 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). Auto correlation function (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 and three representative ages (35, 50 and 70 years, which 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 smoking status, number of comorbidities, BMI, blood eosinophilia and F_{ENO} at baseline screening time. We deleted missing data on adjustment variables listwise.

Statistical analyses were performed in R v4.0.3 (www.r-project.org), using additional packages nlme, splines, ggplot2 [20] and ggeffects [21] to produce the growth charts. p-values <0.05 were considered significant.

Results

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 the Veneto region, Italy, at the time of the analysis. Patients' characteristics at baseline are reported for the whole sample and stratified by sex and age subgroups (table 1, supplementary table S1). Overall 37 patients (58%) were women and 27 (42%) were men, the median age was 52 years and the median BMI was 24 kg·m^{-2}. The majority of patients (79%) had no smoking history (88% of women and 68% of men). Only a small percentage of patients had bronchiectasis (7%, 13% of women and none of the men), autoimmune diseases (5%), interstitial lung disease (3%), dermatitis (3%) or GERD (19%); by contrast, the majority of patients had rhinitis (93%) and atopy (85%). Half of the men did not take OCS at baseline, while the median OCS dose for women was 5 mg·day^{-1}. Eosinophil counts were higher among women (median 650 cells·mm^{-3}) compared to men (median 435 cells·mm^{-3}). No relevant differences existed in general between sexes at baseline for lung function measurements (FEV_1 % predicted, FVC and ACT score).

TABLE 1 Baseline characteristics of patients with severe asthma distinguished by sex and age group (tertiles)

	Overall sample (n=64)	Men			Women			Total men (n=27)	Total women (n=37)
		19–47 years (n=9)	48–56 years (n=6)	57–83 years (n=12)	19–47 years (n=13)	48–56 years (n=14)	57–83 years (n=10)		
BMI (kg·m⁻²) (n=62; NA=2)									
Median (25th–75th centiles)	24.25 (21.8–26.7)	22.2 (20.9–28.7)	25.1 (23.7–25.6)	26.55 (24.3–28.3)	22.82 (19.9–25.8)	22.0 (21.1–25.4)	25.8 (21.9–26.5)	24.8 (23.1–28.4)	22.8 (21.0–26.5)
Smoking history (n=58; NA=6)									
No	46 (79)	6 (67)	3 (50)	8 (80)	11 (85)	10 (100)	8 (80)	17 (68)	29 (88)
Yes	12 (21)	3 (33)	3 (50)	2 (20)	2 (15)	0 (0)	2 (20)	8 (32)	4 (12)
Comorbidities, n (n=64; NA=0)									
Median (25th–75th centiles)	3.0 (2.0–3.0)	3.0 (2.0–3.0)	3.0 (2.3–3.0)	2.0 (1.8–3.0)	3.0 (2.0–4.0)	2.0 (1.0–3.0)	3.0 (2.0–3.8)	2.0 (2.0–3.0)	3.0 (2.0–3.0)
Polyposis (n=62; NA=2)									
No	28 (45)	4 (44)	1 (17)	6 (50)	6 (46)	5 (39)	6 (67)	11 (41)	17 (49)
Yes	34 (55)	5 (56)	5 (83)	6 (50)	7 (54)	8 (62)	3 (33)	16 (59)	18 (51)
Bronchiectasis (n=57; NA=7)									
No	53 (93)	9 (100)	6 (100)	10 (100)	10 (83)	8 (80)	10 (100)	25 (100)	28 (88)
Yes	4 (7)	0 (0)	0 (0)	0 (0)	2 (17)	2 (20)	0 (0)	0 (0)	4 (13)
Rhinitis (n=58; NA=6)									
No	4 (7)	0 (0)	0 (0)	2 (20)	0 (0)	1 (10)	1 (10)	2 (8)	2 (6)
Yes	54 (93)	9 (100)	6 (100)	8 (80)	13 (100)	9 (90)	9 (90)	23 (92)	31 (94)
Atopy (n=59; NA=5)									
No	9 (15)	0 (0)	1 (17)	2 (18)	0 (0)	4 (40)	2 (20)	3 (12)	6 (18)
Yes	50 (85)	9 (100)	5 (83)	9 (82)	13 (100)	6 (60)	8 (80)	23 (89)	27 (82)
Autoimmune diseases (n=58; NA=6)									
No	55 (95)	9 (100)	6 (100)	10 (100)	12 (92)	9 (90)	9 (90)	25 (100)	30 (91)
Yes	3 (5)	0 (0)	0 (0)	0 (0)	1 (8)	1 (10)	1 (10)	0 (0)	3 (9)
Interstitial lung disease (n=58; NA=6)									
No	56 (97)	9 (100)	6 (100)	10 (100)	12 (92)	9 (90)	10 (100)	25 (100)	31 (94)
Yes	2 (3)	0 (0)	0 (0)	0 (0)	1 (8)	1 (10)	0 (0)	0 (0)	2 (6)
Dermatitis (n=58; NA=6)									
No	56 (97)	9 (100)	6 (100)	10 (100)	12 (92)	10 (100)	9 (90)	25 (100)	31 (94)
Yes	2 (3)	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	1 (10)	0 (0)	2 (6)
GERD (n=58; NA=6)									
No	47 (81)	8 (89)	6 (100)	9 (90)	10 (77)	9 (90)	5 (50)	23 (92)	24 (73)
Yes	11 (19)	1 (11)	0 (0)	1 (10)	3 (23)	1 (10)	5 (50)	2 (8)	9 (27)

Continued

TABLE 1 Continued								
	Overall sample (n=64)	Men			Women			Total men (n=27)	Total women (n=37)
		19–47 years (n=9)	48–56 years (n=6)	57–83 years (n=12)	19–47 years (n=13)	48–56 years (n=14)	57–83 years (n=10)		
Biological drugs (n=61; NA=3)									
Mepolizumab	26 (43)	2 (22)	2 (33)	6 (55)	2 (15)	10 (83)	4 (40)	10 (39)	16 (46)
Omalizumab	35 (57)	7 (78)	4 (67)	5 (46)	11 (85)	2 (17)	6 (60)	16 (62)	19 (54)
OCS (mg) (n=64; NA=0)									
Median (25th–75th centiles)	2.5 (0.0–7.2)	0.0 (0.0–5.0)	2.5 (0.0–5.0)	5.0 (3.8–12.5)	5.0 (0.0–5.0)	0.0 (0.0–5.0)	2.5 (0.0–10.0)	5.0 (0.0–10.0)	0.0 (0.0–6.3)
Blood eosinophils (cells·mm^{-3}) (n=61; NA=3)									
Median (25th–75th centiles)	570.0 (300.0–860.0)	500.0 (430.0–680.0)	420.0 (282.5–760.0)	400.0 (272.5–707.5)	720.0 (600.0–960.0)	880.0 (345.0–1080.0)	340.0 (272.5–552.5)	435.0 (275.0–702.5)	650.0 (320.0–1000.0)
F_{ENO} (ppb) (n=61; NA=3)									
Median (25th–75th centiles)	48.2 (28.2–87.5)	41.4 (34.7–45.0)	59.0 (30.3–83.9)	55.4 (29.6–89.3)	37.12 (27.4–92.2)	74.0 (57.0–99.8)	39.66 (20.3–48.0)	45.0 (30.9–79.7)	49.0 (27.1–93.0)
FEV$_1$ t0 (n=63; NA=1)									
Median (25th–75th centiles)	70.0 (59.5–83.5)	69.0 (62.0–76.0)	76.0 (66.0–81.8)	77.0 (66.0–80.0)	85.0 (68.0–93.0)	64.5 (44.5–76.8)	64.0 (59.8–81.5)	73.5 (62.8–80.5)	68.0 (56.0–87.0)
FVC t0 (n=63; NA=1)									
Median (25th–75th centiles)	86.0 (73.0–98.5)	83.0 (65.0–92.0)	92.0 (79.3–109.3)	85.0 (80.5–88.0)	88.0 (69.0–103.0)	75.5 (63.3–94.5)	88.0 (87.3–96.3)	84.0 (77.5–92.8)	88.0 (69.0–103.0)
Tiffeanu t0 (n=62; NA=2)									
Median (25th–75th centiles)	87.5 (77.3–97.0)	83.0 (81.0–91.0)	83.0 (74.3–88.0)	91.0 (84.0–97.5)	97.0 (88.0–101.0)	82.0 (73.0–89.0)	78.5 (75.5–90.0)	87.0 (80.2–94.5)	87.5 (77.0–98.2)
ACT t0 (n=62; NA=2)									
Median (25th–75th centiles)	17.0 (15.0–19.0)	18.0 (15.0–18.0)	16.0 (14.5–20.5)	18.0 (16.0–20.5)	15.0 (13.0–17.0)	17.0 (15.0–19.0)	18.5 (17.0–24.0)	18.0 (15.2–19.0)	17.0 (14.8–19.0)

Data presented as n (%) where not otherwise stated. BMI: body mass index; NA: not applicable; GERD: gastro-oesophageal reflux disease; OCS: oral corticosteroids; F_{ENO}: fraction of exhaled nitric oxide; FEV$_1$: forced expiratory volume in 1 s; t0: baseline visit; FVC: forced vital capacity; ACT: Asthma Control Test.

Outcome measurement trends over visit time

Exploratory data analysis guided the model-building process. Spaghetti plots (figure 1) provided insight for individual's raw data evolution, revealing a nonlinear trend of patients' individual measures of FEV_1 % predicted, Tiffeneau % and ACT score during follow-up distinguished by sex and age. Mean values of FEV_1 % predicted, Tiffeneau % and ACT score at baseline and over follow-up visits for the overall sample are shown in figures 2–4. LMMs with a fixed factor variable for follow-up time (four levels factor) were fitted for each outcome to investigate the responses to biological therapy separately for the subset of men and women. The fitted models included age (years at start of therapy) as an additional predictor. Model parameters provided an estimate of the average growth or decline in FEV_1 % predicted, Tiffeneau % and ACT scores between the sex subgroups in each visit interval. The two subgroups showed different evolution over time (table 2). A significant association between Tiffeneau % levels and age was found for the female subgroup.

Considering the exploratory and the subgroup analysis results, in the overall sample analysis, cubic splines function were included in the implemented LMMs to parameterise individual trajectories over the course of follow-up.

First, a model was fitted assuming an interaction between sex and time of follow-up, which showed no significant evidence for a different treatment response between the two groups of patients (supplementary table S2). Second, a three-way interaction was assumed between sex, time of follow-up and patients' age. Estimated parameters for models with (supplementary table S5) and without (supplementary table S3) covariate adjustment are provided. The final (with covariate adjustment) models for three outcomes were used to estimate age-specific growth/decline curves for men and women, along with 95% confidence intervals (table 3, figure 2).
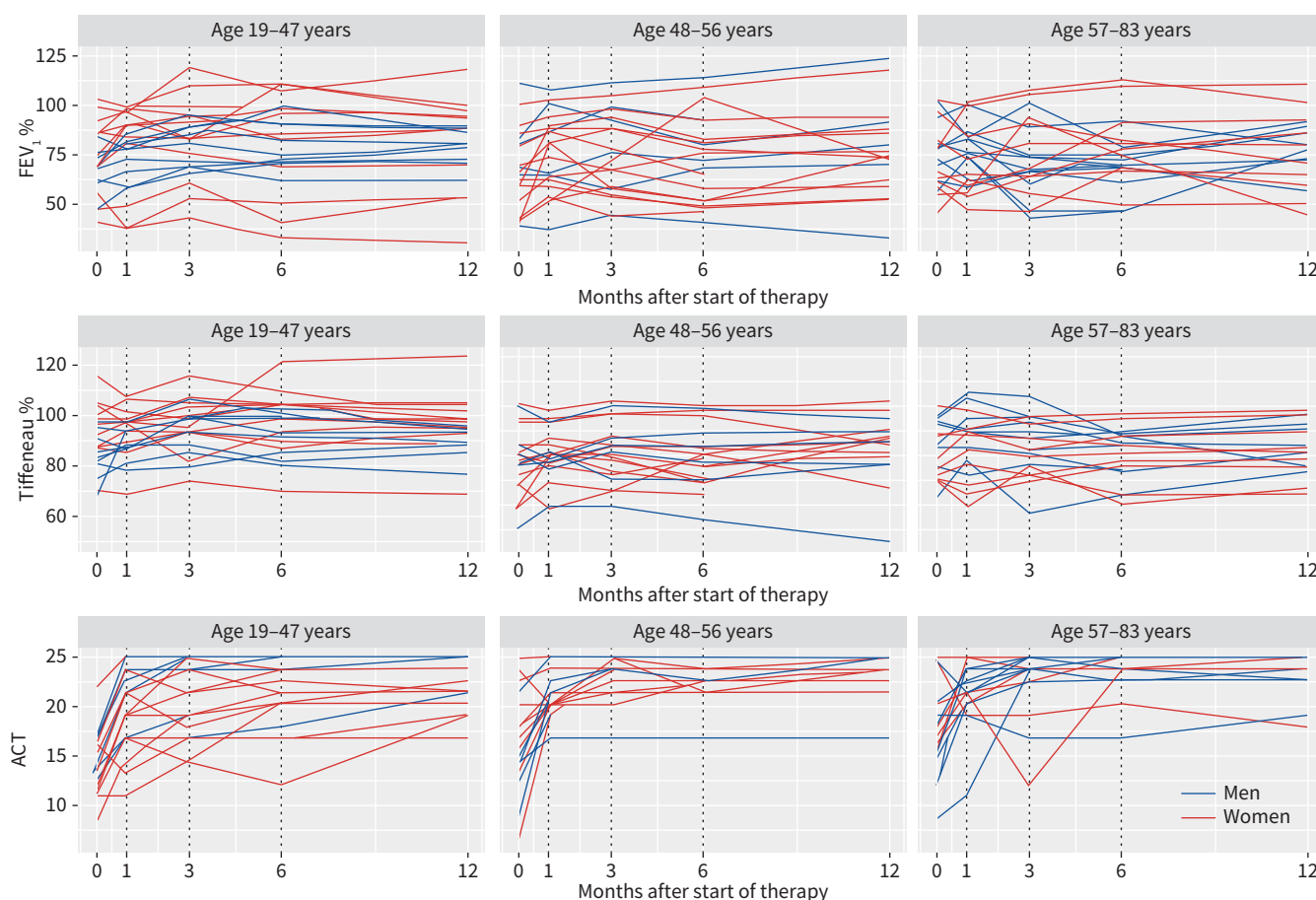
FIGURE 1 Individual raw data trajectories (spaghetti plot) for each outcome measure, divided by age groups (tertiles). FEV_1: forced expiratory volume in 1 s; ACT: Asthma Control Test.
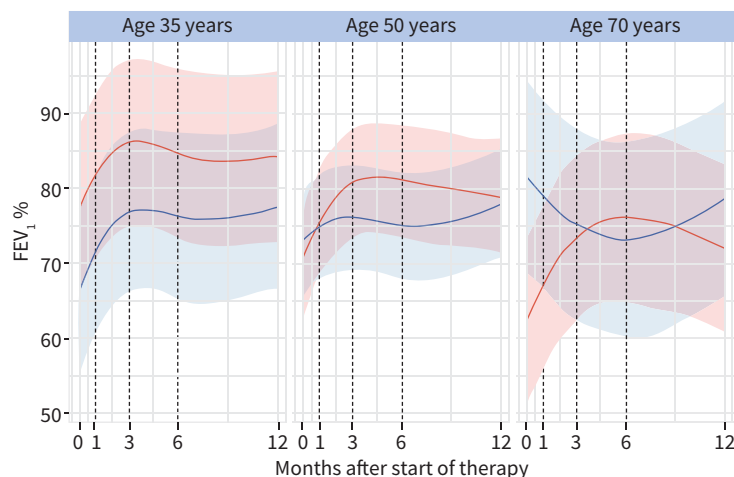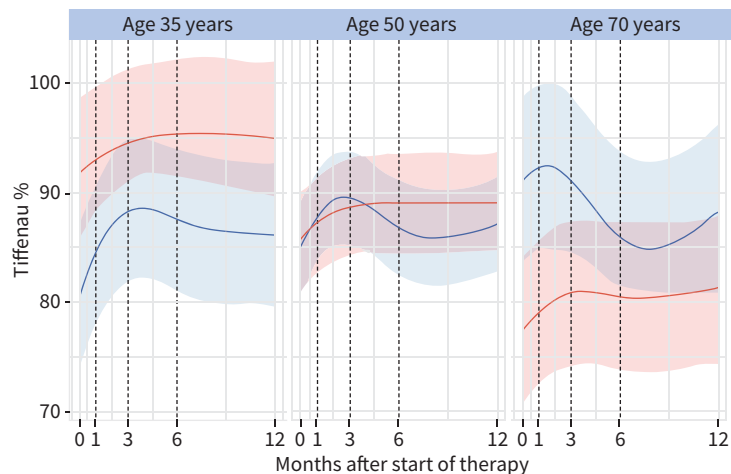
FIGURE 2 Predicted values (lines) and confidence intervals (shaded contours) for forced expiratory volume in 1 s (FEV_1) % of men (blue) and women (pink) at the age of 35, 50 and 70 years old. All the other variables in the model were held constant at their mean values (for numeric vectors) or at their factor's category proportions (for factor variables).

Estimated marginal mean predictions and plots are also presented for different age values (25 years, 55 years, 75 years) (supplementary table S4, figure S3).

### Response to biological drug therapy
OCS

The reduction in OCS daily dose at the 12-month follow-up (t12) in comparison with baseline was explored. Among the 32 subjects regularly taking OCS at the time of biological treatment start, OCS dose was reduced by 82%, on average, from pre to post therapy. There was no clinically relevant difference in the reduction of OCS between men and women (men: mean 0.79 mg, median 1.00 mg, interquartile range (IQR) 0.80–1.00 mg; women: mean 0.85 mg, median 1.00 mg, IQR 0.80–1.00 mg) or among patients' age classes (19–47 years: mean 0.86 mg, median 1.00 mg, IQR 1.00–1.00 mg; 48–56 years: mean 0.82 mg, median 1.00 mg, IQR 0.90–1.00 mg; 57–83 years: mean 0.79 mg, median 1.00 mg, IQR 0.65–1.00 mg). OCS therapy was stopped by 23 patients (72%).

FIGURE 3 Predicted values (lines) and confidence intervals (shaded contours) for Tiffeneau % of men (blue) and women (pink) at the age of 35, 50 and 70 years old. All the other variables in the model were held constant at their mean values (for numeric vectors) or at their factor's category proportions (for factor variables).
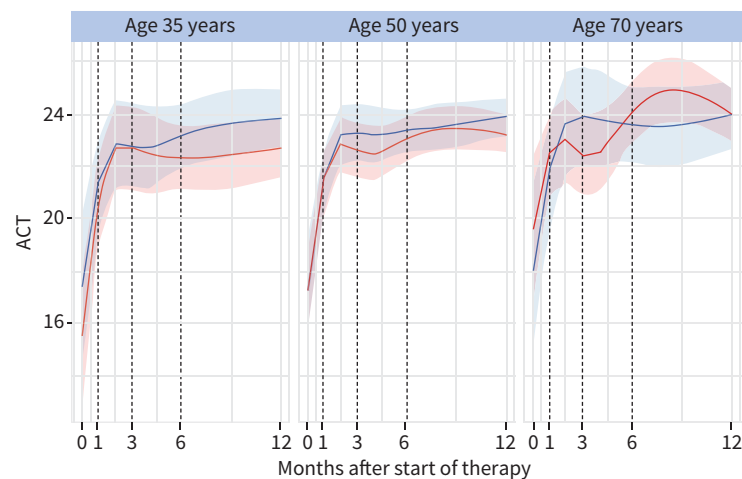
FIGURE 4 Predicted values (lines) and confidence intervals (shaded contours) for Asthma Control Test (ACT) scores of men (blue) and women (pink) at the age of 35, 50 and 70 years old. All the other variables in the model were held constant at their mean values (for numeric vectors) or at their factor's category proportions (for factor variables).

FEV$_1$ % predicted

A first exploration of separate lung function growth/decline rates for men and women showed an improvement in FEV$_1$ % predicted for both subgroups, from start of therapy to end of follow-up (table 2). In women, FEV$_1$ % predicted improved at a higher rate from after the first month of therapy, while in men the highest rate of improvement was at t12. Age was not associated with different mean values of FEV$_1$ % predicted in women or men.

TABLE 2 Estimated parameters for linear mixed models with linear splines fitted on the subset of men and women separately

	Women		Men	
	Value±SE	p-value	Value±SE	p-value
FEV$_1$ %	**n=37; obs=179**		**n=27; 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 %	**n=36; obs=177**		**n=27; 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; obs=158**		**n=22; 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

FEV$_1$: forced expiratory volume in 1 s; t1, 3, 6, 12: visits at 1, 3, 6 and 12 months after treatment start; ACT: Asthma Control Test; obs: number of observations.

TABLE 3 Predictive values of forced expiratory volume in 1 s (FEV_1) %, Tiffeneau % and Asthma Control Test (ACT) for men and women of 35, 50 and 70 years old using linear mixed models with interaction between months after start of therapy (natural cubic splines functions), sex and age

	Men	Women
FEV_1 %[#]		
Age: 35 years		
t0	66.27 (55.36–77.17)	77.41 (66.09–88.73)
t1	71.78 (61.39–82.17)	82.10 (71.26–92.94)
t3	77.03 (66.17–87.89)	86.25 (75.02–97.48)
t6	76.36 (65.39–87.33)	84.79 (73.41–77.17)
t12	77.58 (66.59–88.57)	84.33 (72.94–95.73)
Age: 50 years		
t0	72.92 (65.73–80.11)	70.95 (63.46–78.43)
t1	74.96 (68.11–81.80)	75.75 (68.55–82.95)
t3	76.31 (69.16–83.46)	80.85 (73.42–88.28)
t6	75.04 (67.80–82.27)	81.11 (73.59–88.63)
t12	78.04 (70.80–85.29)	79.11 (71.58–86.63)
Age: 70 years		
t0	81.79 (68.74–94.83)	62.33 (51.13–73.53)
t1	79.19 (66.73–91.64)	67.29 (56.64–77.94)
t3	75.36 (62.43–88.29)	73.65 (62.56–84.74)
t6	73.27 (60.15–86.39)	76.20 (64.93–87.48)
t12	78.66 (65.52–91.79)	72.13 (60.85–83.42)
Tiffeneau percentage[¶]		
Age: 35 years		
t0	80.48 (73.97–87.00)	91.98 (85.2–98.77)
t1	84.59 (78.34–90.84)	93.13 (86.58–99.68)
t3	88.43 (81.99–94.88)	94.67 (87.95–101.4)
t6	87.54 (80.97–94.10)	95.50 (88.67–87.00)
t12	86.19 (79.62–92.77)	95.13 (88.28–101.97)
Age: 50 years		
t0	85.03 (80.73–89.33)	85.71 (81.21–90.21)
t1	87.92 (83.8–92.05)	87.15 (82.79–91.51)
t3	89.63 (85.38–93.88)	88.76 (84.29–93.22)
t6	86.83 (82.49–91.16)	89.09 (84.56–93.62)
t12	87.12 (82.78–91.46)	89.16 (84.63–93.70)
Age: 70 years		
t0	91.09 (83.26–98.91)	77.34 (70.63–84.04)
t1	92.37 (84.84–99.90)	79.18 (72.74–85.61)
t3	91.22 (83.48–98.97)	80.86 (74.23–87.50)
t6	85.88 (78.01–93.76)	80.54 (73.78–87.29)
t12	88.35 (80.47–96.24)	81.21 (74.44–87.97)
ACT score[+]		
Age: 35 years		
t0	17.24 (14.37–20.12)	15.52 (12.84–18.20)
t1	21.38 (19.65–23.12)	20.72 (19.05–22.39)
t3	22.79 (21.16–24.41)	22.73 (21.15–24.30)
t6	23.18 (22.01–24.35)	22.33 (21.13–20.12)
t12	23.82 (22.76–24.87)	22.72 (21.62–23.83)
Age: 50 years		
t0	17.53 (15.62–19.44)	17.25 (15.54–18.96)
t1	21.59 (20.42–22.75)	21.49 (20.39–22.58)
t3	23.28 (22.18–24.37)	22.6 (21.57–23.64)
t6	23.35 (22.54–24.16)	23.09 (22.28–23.91)
t12	23.88 (23.15–24.62)	23.27 (22.51–24.02)
Age: 70 years		
t0	17.92 (14.7–21.13)	19.56 (16.82–22.29)
t1	21.86 (19.86–23.86)	22.51 (20.84–24.17)
t3	23.94 (22.06–25.82)	22.44 (20.88–24.00)
t6	23.58 (22.16–25.00)	24.11 (22.97–25.25)
t12	23.97 (22.66–25.28)	23.99 (22.95–25.02)

Data are presented as predicted values (95% CI). Models were adjusted for biological drug, smoking history, body mass index (BMI), number of comorbidities, fraction of exhaled nitric oxide (F_{ENO}) and blood eosinophilia, held constant at their mean values or at their category's proportions in case of factors. t0, 1, 3, 6, 12: visit at baseline, 1, 3, 6 and 12 months after treatment start. [#]: adjusted for N comorbidity=2.65, BMI=24.5, F_{ENO}=61.7, blood eosinophilia=642.7; [¶]: adjusted for N comorbidity=2.65, BMI=24.6, F_{ENO}=61.7, blood eosinophilia=654.7; [+]: adjusted for N comorbidity=2.63, BMI=24.8, F_{ENO}=63.8, blood eosinophilia=654.8.

Whole cohort data were analysed in the final set of LMMs (introduced in the section 'Statistical analysis'), using natural cubic splines for nonlinear growth/decline parameterisation over time of follow-up (supplementary table S5). In young men, FEV_1 % outcome was predicted to improve in the first 3 months (from 66.3%, 95% CI 55.4–77.2% to 77.0%, 95% CI 66.2–87.9%), reaching a maximum value (77.6%, 95% CI 66.6–88.6%) between t6 and t12, while young women, on average, reached a maximum improvement of FEV_1 % predicted at t3 (from 77.4%, 95% CI 66.1–88.7% to 86.3%, 95% CI 75.0–97.5%) (figure 2, table 3). Middle-aged men and women reached comparable mean predicted values of FEV_1 % at t12 (men: 78.0%, 95% CI 70.8–85.3%; women: 79.1%, 95% CI 71.6–86.6%), with a comparable trend. Older men had an opposite trend in FEV_1 % predicted value when compared to women of the same age. Older men showed no increase in FEV_1 % predicted (from 81.8%, 95% CI 68.7–94.8% at baseline to 78.7%, 95% CI 65.5–91.8% at t12), while women, who started from lower values at baseline (62.3%, 95% CI 51.1–73.5%), showed an increase at t12 (72.1%, 95% CI 60.9–83.4%).

Tiffeneau

Significant improvement in Tiffeneau % values were evidenced from baseline to the end of follow-up for both men and women; men had a higher rate of improvement at t1 and t3 and a decline in the following visit times, while the rate of improvement in women was higher at t12 (table 2). Women also had lower mean values of Tiffeneau % with increasing age, which were not observed in men. Final LMMs with natural cubic splines for the whole cohort data showed a reduction of Tiffeneau % function for women as their age increased. This was not observed in men. Over months of follow-up, mean predicted values for women increased from 92.0% (95% CI 85.2–98.8%), 85.7% (95% CI 81.2–90.2%) and 77.3% (95% CI 0.6–84.0%) in the three age groups at t1, respectively, to 94.7% (95% CI 88.0–101.4%), 88.8% (95% CI 84.3–93.2%) and 80.9% (95% CI 74.2–87.5%) at t3. Values stayed stable until the end of follow-up. Young and middle-aged men had the same increase over the first 3 months from 80.5% (95% CI 74.0–87.0%) and 85.0% (95% CI 80.7–89.3%) to 88.4% (95% CI 82.4–94.4%) and 89.6% (95% CI 85.3–93.9%), respectively. Predicted values decreased from t6 to t12. Older men had an improvement only in the first month and then decreased until t12. At t12, young and middle-aged men showed higher values (95.1%, 95% CI 88.3–102.0% and 89.2%, 95% CI 84.6–93.7%, respectively) as compared to baseline, while older men had lower values (88.4%, 95% CI 80.5–96.2%) when compared to baseline.

ACT score

Raw data exploration showed that ACT values for both men and women in the cohort had decreasing variability over the follow-up period (supplementary figure S1).

Significant improvement in ACT scores was evidenced at all visit times when exploring male and female growth rates separately (table 2). No association was found between age variable and ACT score for any of the subgroups.

Final LMMs with natural cubic splines exploring the whole cohort data showed for both men and women, regardless of age, a significant increase of mean predicted ACT scores, from 17.5 (95% CI 15.8–19.2) and 17.6 (95% CI 15.6–19.5) at t1, respectively, to 23.3 (95% CI 22.6–24.1) and 23.9 (95% CI 23.2–24.6) at t12. Women had higher ACT scores with increasing age. This was not observed in men. Older women showed a first increase from baseline to t3 and a second increase from t6 to t12 (figure 4).

Discussion

Our study described the trend of clinical and functional parameters in severely asthmatic men and women receiving biological treatment over a 12-month follow-up period. No significant differences in terms of lung function, patient-reported asthma control, exacerbation rate or daily OCS dose were observed by sex within the study timeframe. When including age stratification, lung function parameters (FEV_1 and Tiffeneau index) in older men were significantly lower than in older women. A similar trend was not observed in terms of ACT score, which improved in both men and women in the same age range. No other significant differences were detected when stratifying by age.

When investigating the response to a biological treatment for severe asthma, sex and age are usually considered to be 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 analysing potential predictors of response to biologicals, including sex and age, which in those cases do not seem to have any relevance [11, 22]. Only one study described female sex as a determinant of better response to omalizumab treatment in adults [23].

Our study aimed to explore as a primary outcome the potential interaction between sex, age and the response to biological 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 F_{ENO} values and blood eosinophilia, in order to explore the "weight" of sex first and age on biological treatment clinical outcomes. As shown in figures 2–4, an improvement in FEV_1 % predicted, Tiffeneau % and ACT score was observed in both men and women, particularly at t1 (1 month after the start of biological therapy). The subsequent time-point assessment revealed a less marked improvement, but higher values in comparison with baseline were still observed.

A combined action of both biological 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, clinical control could be sustained by biological therapy, and this might explain the minimal deflection in clinical control. In fact, in the considered follow-up period of 12 months, an 82% reduction in the oral steroid daily dose was registered, regardless of sex, and 72% of patients completely discontinued steroid therapy.

Although generally speaking, in clinical practice sex does not seem to determine treatment response, some sex- and age-related peculiarities deserve to be highlighted.

Considering the analysis for the two sex subsets separately (table 2), asthmatic women experienced a faster increase in FEV_1 % predicted (which was already significant at t1) that was maintained at each consecutive time point, while men had an improvement in FEV_1 % predicted that was only evident 12 months after treatment start. This difference was better detailed in the analysis for the overall sample, where a three-way intersection has been parametrised between time, sex and age variables. In particular, older men seemed to have no improvement in FEV_1 % predicted over time. By contrast, women of the same age had an improvement in FEV_1 % during follow-up visits (figure 2, table 3). Similarly, older men showed no increase in the Tiffeneau % values, as compared to women of the same age.

With regards patient self-reported outcomes, ACT scores increased significantly in both men and women of all age classes at each time point. Only women showed significantly higher mean values as age increased. There was comparable evolution of ACT scores over time (table 3, figure 2), except in older women, who showed a non-monotone ACT improvement.

A weak correlation between lung function and patient-reported outcomes has already been described elsewhere [24]. Age and sex have been highlighted as determinants of asthma control perception [25]. In particular, discrepancies between lung function and ACT score seem to be more relevant in older people. Distinguishing by sex, women have been described to be more sensitive to changes in lung function and to excessively amplify them when reporting subjective evaluation of asthma control as compared to men.

From a pathophysiological perspective, sex discrepancy in older people may be related to the physiological sexual hormone variations that occur with ageing. It is well known that T-helper 2 (Th2)-mediated airway inflammation is sustained and amplified by oestrogens (although the mechanisms are not completely clear) and downregulated by androgens, which play a crucial protective role in type 2 bronchial inflammation [4, 5, 26, 27]. In animal models, testosterone modulates type 2 innate lymphoid cell proliferation, and dehydroepiandrosterone (DHEA), a hormone upstream of testosterone that is produced by the adrenal glands as well as by the gonads, decreases levels of serum eosinophils, interleukin (IL)-5, IL-4 and interferon-γ [2]. In addition, it has been demonstrated in men that 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, known as andropause [28]. Studies have reported that free testosterone declines by 1.2% per year and DHEA by 3.1% per year, with the incidence of hypogonadism increasing to about 20% in men over 60, 30% in men over 70 and 50% in men over 80 years of age [29–31]. Conversely, with the onset of menopause, by the mid-sixth decade of life all women experience a dramatic decline in oestrogens levels [28]. The above-mentioned evidence may provide a potential explanation for the lack of improvement or, in some cases, the worsening in lung function observed in older men after treatment with biological drugs. In particular, the age-related reduction of androgens and consequent loss of their protective role characterising andropause may account for the parallel increase with age of bronchial inflammation and for the limited response to therapeutic interventions. Likewise, the improvement in treatment response in older 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 allow the exploration of a very specific group of asthma patients, thus limiting the possible selection bias. However, this study is particularly powered by the use of models that included the set of available patient measurements over time, giving not only a quantitative evaluation of the outcomes but also a qualitative estimation of their evolution during follow-up.

To the best of our knowledge, no studies have previously analysed the relevance of sex and age in the clinical response to biologicals in severe asthma as the primary outcome. Furthermore the "weight" of sex has been explored by excluding potential confounding factors, such as the specific biological treatment, smoking history, BMI, number of comorbidities, baseline F_{ENO} and blood eosinophilia. However, the external validity of our findings needs to be confirmed in larger studies that also include more biological drugs.

Conclusions

According to our findings, sex does not act as a determinant of treatment response to biologicals in people with severe asthma 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, older women showed a significantly higher lung function than 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 nor age should limit biological treatment prescription, once the eligibility criteria for that specific therapy are satisfied.

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