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Using the six-minute walking test to assess the clinical response to mepolizumab and conventional therapy in severe eosinophilic asthma

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ABSTRACT

BACKGROUND: Severe asthma limit exercise to avoid respiratory symptoms.

OBJECTIVE: To investigate the role of 6-Minute Walking Test (6MWT) in severe asthma.

METHODS: Consecutive patients with severe eosinophilic asthma were enrolled. 6MWT was performed before and after 12 months. Inhaled therapy dose, oral corticosteroids (OCS) dose, pulmonary function tests (PFT), eosinophils blood count, fractional exhaled nitric oxide (FeNO), asthma control test (ACT) and asthma quality of life (AQLQ) questionnaires were also recorded.

RESULTS: Of the 22 patients enrolled, 13 were treated with mepolizumab 100mg/month in addition to conventional therapy and 9 only with conventional therapy. The majority of the patients were treated with high-dose inhaled corticosteroids (ICS)/long-acting β-agonists (LABA)/long-acting muscarinic receptor antagonists (LAMA), while approximately half were on continuous OCS.

After 12 months, a significant improvement of the PFT (%FEV1 and %FEF25-75, both $p<0.001$; %FVC, $p<0.01$) and clinical-laboratory parameters (Eosinophils, FeNO50, ACT and AQLQ, $p<0.001$) were observed only in the mepolizumab group. No significant changes in the proportion of patients using continuous OCS and high dose ICS/LABA/LAMA were observed in both groups ($p>0.05$).

By paired comparisons, statistically significant improvements of the mean 6MWT distance (6MWD) were observed in mepolizumab $p<0.001$) and conventional-therapy groups ($p<0.01$), while dyspnea Borg scale, heart rate, %Oxygen saturation, systolic and diastolic blood pressure did not. 6MWD showed significant direct correlations with ACT ($r=0.5998$, $p<0.001$), AQLQ ($r=0.3978$, $p=0.009$) and FEF25-75% ($r=0.3589$, $p=0.017$).

CONCLUSIONS: 6MWT could complement the severe asthma assessment and be relevant in the evaluation of the objective response to treatment, including biologic therapies like mepolizumab.

INTRODUCTION

The 6-minute walking test (6MWT) is a simple and inexpensive test that evaluates physical performance and walking capacity(1-3). It was developed by the American Thoracic Society and it was officially introduced in 2002, coming along with a comprehensive guideline(4). The test was initially designed to help in the assessment of patients with cardiopulmonary issues. Gradually, it was introduced in other conditions, such as asthma and chronic obstructive pulmonary disease (COPD). 6MWT evaluates the functional capacity and it provides valuable information regarding all the systems during physical activity, including pulmonary and cardiovascular systems. Test results are related to day-to-day physical activity and can also be assumed to represent a direct measure of impaired quality of life(5).

Walking tests are suitable in patients that otherwise have difficulty in performing standard exercise tests, and 6MWT has been mainly used in patients with chronic heart failure, pulmonary diseases (i.e. COPD, sarcoidosis, pulmonary fibrosis, pulmonary arterial hypertension) and obesity to evaluate prognosis and therapeutic response(5-10). For example, in patients with severe COPD, initial 6MWT was predictive of survival. Dajczam et al. demonstrated that overall survival at three years was only 58% and was especially poor (34%) in COPD patients with low (<150 m) initial walk distance(11). An increase in the distance walked indicates improvement in basic mobility. At least in severe COPD patients, the most updated minimal important difference of exercise tests is about 25 meters, which means that in amputee rehabilitation post training a difference of at least 45 meters should be observed for the 6MWT to ensure a reasonably substantial change(12). However, 6MWT cannot give a pathophysiologic insight into the exercise intolerance/dyspnea of patients, unlike other tests such as the cardiopulmonary exercise testing, which is anyway more time consuming and expose the patients to some risks from a cardiovascular standpoint.

In contrast to other diseases studied by 6MWT, we are not aware of any studies to date that have specifically evaluated the utility of this test in assessing the impact of biological therapy in

patients with severe asthma. About 5–10% of patients with asthma are deemed to have severe disease(13) and severe asthma represents up to 50% of total asthma-related healthcare costs.

Severe asthma can be divided into several phenotypes, of which severe eosinophilic asthma is among the most studied(14). It has a great impact on the quality of life (QoL) of patients and their families. The magnitude of this morbidity is affected by several personal factors, including age. The use of biologics has shown some promising effects on the QoL of patients with severe uncontrolled asthma(15).

Moreover, severe asthma patients have some functional similarities with COPD patients, often presenting with non-reversible bronchial obstruction. In addition, the frequent use of systemic steroids in these patients can lead to muscle wasting and reduced physical activity and functional capacity. These similarities thus represent a rational approach for proposing the 6MWT as an additional tool to evaluate the impact of biologic agent therapy in severe asthma.

In this study, we performed a prospective analysis of the 6MWT in subjects with severe eosinophilic asthma who conducted the test as part of their clinical evaluation prior to and after a 12 month-treatment with the biological agent mepolizumab, a human monoclonal antibody directed against IL-5, while comparing these findings to an historical control group with severe eosinophilic asthma treated only with conventional therapy. We also wanted to evaluate the sensitivity to change of the 6MWT parameters in the whole group, and to test for correlations of the most relevant ones with specific asthma scores and functional features.

MATERIALS AND METHODS

Study Setting and Participants

This was a single center study conducted in an accredited outpatient Severe Asthma Center (Fondazione Poliambulanza Hospital Institute, Brescia, Italy) between December 2019 and May 2022. Consecutive adult patients with severe asthma diagnosed according to the European

Respiratory Society (ERS)/American Thoracic Society (ATS) criteria (13) where prospectively collected. Due to COVID-19 pandemic, after the first patients were enrolled the service was temporarily suspended and even after the first two waves of COVID-19 pandemic, the 6MWT was not regularly performed in Poliambulanza due to safety reasons. Fifty patients were evaluated in the prescreening visit, but only three patients were enrolled and completed the two 6MWT 12 month apart (+/-1 month) until March 2021, therefore most of the patients of this study were enrolled between March 2021 and May 2021, then followed for 1 year until May 2022.

All the patients already had a diagnosis of severe eosinophilic asthma that was confirmed by the pulmonologist of the Poliambulanza Hospital (Severe Asthma Clinic), that have initially evaluated the patient before enrollment (screening visit) and after 4 weeks (visit T0). Before screening visit and visit T0, inhaled therapy was optimized, and comorbidities and treatment compliance checked by the treating physician. Therefore, at least 4 weeks of asthma therapy after a structured medical assessment was done by every patient. The first 6MWT was done at visit T0.

Initial patient assessment was identified with standard investigations such as familiar, personal, and pathological medical history, pulmonary function tests (PFT), eosinophil count, fractional exhaled nitric oxide (FeNO), skin prick tests serum specific IgE, and high-resolution computer tomography.

The asthma control test (ACT) questionnaire was used to measure the degree of asthma control (scores range from 5 (poor control of asthma) to 25 (complete control of asthma), with higher scores reflecting greater asthma control; an ACT score >19 indicates well-controlled asthma), and the asthma quality of life questionnaire (AQLQ) to evaluate the disease-specific health-related QoL (scores range 1-7, with higher scores indicating better quality of life). Patients were also studied to assess the presence of nasal polyposis and/or gastroesophageal reflux.

To avoid confounding effect of smoke, current smokers were excluded from this study, as well as patients engaged in exercise training programs prior to the study. Patients with concomitant upper or lower respiratory tract infection during the 4 weeks before the screening visit or T0 visits were

also excluded, as well as patients fulfilling the criteria for asthma/chronic obstructive pulmonary disease overlap syndrome were excluded.

We compared these results with consecutive severe eosinophilic asthma patients not treated with biologic therapy (this cohort was collected before the AIFA approval of Mepolizumab for asthma), enrolled between December 2013 and December 2014, and followed up by the same pulmonologist at the Poliambulanza Hospital (Severe Asthma Clinic). Only the patients with 6MWT performed at T0 and T1 were included, as the study cohort, and were evaluated with the same structured clinical assessment, and asthma and quality of life scores. The number of patients of the control group is therefore determined by the criteria chosen, to ensure comparability with the mepolizumab group, i.e. to guarantee the same asthma features, age, sex and baseline features in the two groups.

All the patients enrolled underwent to the 6MWT in the days immediately preceding the start of the observation (and the start of biological agent in the mepolizumab group) (T0) and 12 months after starting observation (T1), along with physical examination, blood eosinophil count determination, spirometry, FeNO, ACT and AQLQ questionnaires.

This study was approved by the local Institutional Review Board of Poliambulanza Brescia and was conducted in accordance with the amended Declaration of Helsinki. All patients gave written informed consent for their data to be stored electronically.

The study was conducted according to STROBE guidelines (STrengthening the Reporting of Observational Studies in Epidemiology) for cohort, case-control, and cross-sectional studies.

Treatments

Thirteen patients have been treated with subcutaneous mepolizumab 100mg/4 weeks in addition to conventional, non-biologic therapies for asthma, while nine patients were treated with conventional therapies only.

Inhaled drug therapy dose and oral corticosteroid (OCS) therapy dose were collected at T0 and T1. When possible, these were gradually reduced or discontinued, if asthma symptoms were under control, according to GINA guidelines.

Six-minute walking test (6MWT)

Subjects were instructed to walk at their own maximum walking speed in a long hospital corridor with indicated turning points. Walking distance after 6 minutes (6-minute walking distance, 6MWD) was measured. Heart rate (HR) per minute was measured by a HR monitor after 5 min of rest before the test and immediately after the test. The instrument used to perform the 6MWT was the MIR (Medical International Research) Spirodoc ® (equipped with USB connection).

Pulse oximeter specifications: SpO2 measurement: 0-99% (SpO2 accuracy: ±2% between 70-99% SpO2); pulse rate measurement: 30-254 beats per minute (pulse rate accuracy: ±2beats per minute or 2%, whichever is greater).

Pulse oximeter measured parameters: SpO2 [Basal, Min, Max, Mean], Pulse Rate [Basal, Min, Max, Mean], T90 [SpO2<90%], T89 [SpO2<89%], T88 [SpO2<88%], T5 [ΔSpO2>5%], Δ Index [12s], SpO2 Events, Pulse Rate Events, Bradycardia, Tachycardia], Number of Steps, Movement [VMU].

6MWT parameters collected: O2-Gap, Estimated Distance, Distance Walked, Theoretical Distance [Min, Standard], TΔ2 [SpO2≥2%], TΔ4 [ΔSpO2≥4%], Recording Time, Time [Rest, Walk, Recovery], Desaturation Area/Distance, Borg Dyspnea [Start, End, Diff.], Borg Fatigue [Start, End, Diff.], Blood Pressure [Systolic, Diastolic], Oxygen Administered.

The test was conducted according to the ERS/ATS international standard guidelines(17).

Modified Borg Dyspnea Scale

The American Thoracic Society's 2002 guidelines suggest the modified Borg scale as an aid for the 6MWT, enabling the evaluation of the degree of respiratory discomfort in terms of determinations

of subjective rates, according to the perception of the individual. This is a vertical scale quantified from 0 to 10, in which 0 represents no symptoms and 10 represents maximum symptoms, providing an individual measurement of the intensity of the exercise(18).

All the patients received instructions for the purpose of the scale, how it would be applied, and had time to observe it and adapt to the scale's expressions and numbers. At the beginning and at the end of the 6MWT, the scale was shown to the patient, and the patient was asked to measure the perception of the intensity of the dyspnea.

Statistical analysis

Data are summarized using percentages, means and standard deviations or median and 25-75% interquartile range. Variations in time of the quantitative characteristics, 6MWT and other cardiovascular parameters were assessed using a Student's t-test or a non-parametric Wilcoxon test for paired data, according to the results of the Shapiro–Wilk test of normality on the differences. Moreover, for quantitative characteristics the 95% confidence intervals for the difference of the means (or of the medians) are reported. Variations of the dichotomous variables were assessed with the McNemar's chi-squared test in a two-dimensional contingency table. Statistical significance was set at $P < 0.05$. Wilcoxon signed-rank test was used to analyze paired data before and after the observation time. Spearman's method was used to test for possible correlations between the studied variables.

Data were analyzed using the statistical software R and GraphPad Prism (San Diego, California).

RESULTS

We included 22 patients with severe eosinophilic asthma that undergo to a 6MWT and complete functional and clinical respiratory assessment at baseline and after 1 year of observation, 13 patients in the group treated with mepolizumab and 9 patients in the group treated only with conventional therapies (i.e. not treated with biologic therapy). There was no difference at baseline

between cases and controls in demographic, clinical features, laboratory, pulmonary function test and asthma treatment before the observation period (**Table I**).

In the mepolizumab group, the mean age at enrollment was 53.0±10.4 years, while the age at asthma diagnosis was 35.2±11.3 years. At enrollment, BMI kg/m^2 was 27.9±7.1. Mean eosinophil count at enrolment was 548.7±216.9/mmc, 23% of the patients had nasal polyposis, 23% had gastrointestinal reflux disease (GERD), 31% had bronchiectasis. The 92% the patients were treated with high-dose inhaled corticosteroids (ICSs)/long-acting β-agonists (LABAs)/long-acting muscarinic receptor antagonists (LAMAs), while 46% were on continuous OCS. All the 13 patients of the mepolizumab group started the treatment with mepolizumab 100mg/4 weeks at enrollment, fulfilling the AIFA (Agenzia Italiana del Farmaco) prescribing criteria.

After 1 year of observation, in the mepolizumab group, a significant mean improvement of the PFT (%FEV1 CI95%: 4.6;12.4 and %FEF25-75 CI95%: 8.5;19.5, both $p<0.001$; %FVC CI95%: 1.8;6.5, $p<0.01$), asthma and quality of life scores (ACT CI95%: 5.0;10.2, $p<0.001$; and AQLQ CI95%:0.6;1.8, $p<0.001$), blood eosinophils and FeNO (eosinophil blood counts CI95%: -555.8;-322.9; FeNO 50 CI95%:-16.4;-2.9, $p<0.001$) were observed. In contrast, it has been observed a mean significant improvement only in ACT, AQLQ, and FeNO in the conventional therapy group, without pulmonary function test nor eosinophil level modifications (**Table II**).

No significant modification in the proportion of patient using continuous OCS, inhaled therapy (ICS/LABA/LAMA) and body mass index (BMI) were observed in both groups ($p>0.05$) (**Table II**). None of the patients had at baseline or developed between T0 and T1 new cardiovascular or musculoskeletal issues that could have impaired the results of the 6MWT, while the patients denied a modification in physical habits during the observation period.

When focusing on mean 6MWT parameters (**Table III**), in the mepolizumab group a statistically significant improvement of the 6MWD (from 438.2±46.2 to 506.8±57.7 meters, $p<0.001$) and mean %SpO2 (from 95.0±1.2% to 96.0±1.1%, $p=0.006$) were observed. In the conventional therapy group, there was also the improvement in the mean 6MWD (from 450.3±27,5

to 506.0±41.6 meters, p=0.004) and no significant change in mean %SpO2. There were some improvements in the mean values of Dyspnea Borg Scale in both groups at T1 as compared to T0, while there were no statistically significant changes for heart rate, systolic and diastolic blood pressure in both groups.

By paired comparisons, 6MWD show a stronger improvement ($p<0.001$) than oxygen saturation in ambient air ($p<0.01$) in the mepolizumab group (**Figure 1A**), while all the other parameters did not improve. Importantly, the SpO2 accuracy of the instrument use was greater than the mean difference measured (±2%), therefore indicating that this difference may be due to the case. In the control group, only the 6MWD improved during observation ($p<0.01$) (**Figure 1B**).

The improvement in the 6MWD in the mepolizumab group was observed in all the patients, and in 11 out of the 13 patients this improvement was grater then 45 meters, and in 13 out of the 13 greater than 25 meters, two recognized cut-offs used for COPD (**Figure 2A**), with an overall mean 6MWD of 69±25 (range: 27-106) meters. In the conventional therapy group, in 6 out the 9 patients the 6MWD was greater than 45 meters, and in 8 out of 9 patients was greater than 25 meters, with an overall mean 6MWD of 56±32 (range: 3-113, compared to mepolizumab group p=0.594).

No correlations were observed between the Δ6MWD (value at T1 – value at T0), Δ asthma symptom score (as assessed by ACT), Δasthma-related quality of life score (as assessed by AQLQ), and Δfunctional parameters (FEV1%, FVC%, FEV1/FVC%, FEF25-75%) (**Supplementary Table I**). However, when the crude 6MWD values were pulled together (T0 +T1, n=44) and correlated with the crude values of the same asthma and quality of life scores and functional parameters, significant, direct correlations were observed between 6MWD and ACT, AQLQ, and FEF25-75% (**Supplementary Table I, Figure 2B-D**).

DISCUSSION

Here we showed that in severe asthma patients, 6MWD is a good parameter to complement the assessment of asthma and to evaluate severe eosinophilic asthma, showing sensitivity to change after asthma treatment, and good correlations with asthma symptoms (as assessed by ACT), asthma-related quality of life (as assessed by AQLQ), and small airway dysfunction (as assessed with FEF25-75%). In addition, 6MWD improved after 12-month of observation in our relatively small but very well characterized cohort of 22 patients with severe asthma (13 patients treated with mepolizumab, 9 with conventional therapy), in contrast with the other cardiorespiratory parameters measured during the 6MWT. Notably, the improvement was observed in 100% the patients treated with mepolizumab, and in all the improvement was clinically significant (i.e. > 25 meters) according to published cut off used for COPD (12). The mepolizumab group has a major benefit from the treatment as compared to patients treated with conventional therapy (as expected), especially at a pulmonary function level: functional, clinical and laboratory parameters improved after mepolizumab, i.e. spirometry measurements, eosinophil cell counts, FeNO, ACT and AQLQ, indicating that the 6MWD improvement was paralleled by a functional and disease-related biomarkers improvement, along with the improvement of the quality of life. Taken altogether, our data proved the concept that the 6MWT can be relevant for the objective evaluation of treatment response of severe asthma.

Exercise capacity is associated with symptoms and health-related quality of life in severe asthma. Indeed, dyspnea on exertion is the most reported symptom described patients with uncontrolled asthma, limiting the everyday life activity(19, 20,21).

Evidence indicates that patients with asthma limit exercise and healthy lifestyle activities to avoid respiratory symptoms. This self-imposed decrease in activity may predispose them to long-term general health risks(22).

A group of asthmatic patients reports exercise intolerance leading to limitations in daily life activities as the most prominent symptoms, rather than the wheezing attacks, severely impacting the quality of life(23). This leads to a reduction in physical activity in patients with severe asthma,

which has been scantly reported in these patients(24). In fact, an objective quantification of physical activity in adult patients with stable asthma of different severities has been rarely evaluated, its association with airway physiology is currently lacking. Several studies have recently shown a close link between exercise-related respiratory symptoms and small airway dysfunction, even in the presence of normal respiratory function (FEV 1 and FEV1/FVC)(23,25-28).

Significantly, of the correlations between 6MWD and functional parameters, asthma symptoms and quality of life, only FEF25-75%, ACT (the strongest correlations) and AQLQ resulted significant. To us, this suggests that the relationship between the 6MWD could be linked to the function of small airways, that has been shown to be linked to exercise induced asthma(33,34), correlating with asthma symptoms and quality of life. Given that the ultimate goal of the therapy for asthma is to achieve patient well-being, 6MWD might be a way to objectively assess the "overall" status of these patients. From a treatment perspective, the advances in phenotyping and endotyping severe asthma gave us new insights on personalized therapies, i.e. biological agents for severe asthma. Biological therapies target cytokines/mediators (e.g. Interleukin-5) or immunoglobulins (e.g. IgE) and more in general cells and pathways involved in the pathophysiology of asthma, counteracting the inflammation at the respiratory tract and thus impacting the natural course of asthma, i.e. reducing the exacerbation and avoiding airway remodeling. In addition, they usually allow a reduction in the oral and inhaled corticosteroids doses, avoiding the short- and long-term adverse effects connected to their use, improving their quality of life and socio-economic costs(16). Multiple randomized controlled trials on severe eosinophilic asthmatic patients demonstrated the efficacy of mepolizumab in reducing blood eosinophilia. This has been associated with a reduction of the rate of severe exacerbations and oral corticosteroid use while improving asthma control and lung function(16). In contrast, an improvement in exercise was never investigated in the context of a clinical trial. In our cohort, the reduction of eosinophils did not correlate with the change in 6MWTD (not shown).

Carpagnano et al. investigated the physical activity in two groups of patients with severe asthma analyzing the changes occurring in 30 patients treated with biologic therapies (omalizumab and mepolizumab) and 20 patients who underwent traditional treatment over 6 months(29). This study represents a pioneer trial, since it is the first showing a positive correlation between biologic drug therapy and daily physical activity (recorded with a specific monitor) compared to the effects of traditional therapy in patients with severe asthma.

These results are in line with those of our prospective study, in which we used 6MWD as surrogate of clinical activity to measure the impact of mepolizumab in asthma. Our findings showed the mean variation of 6MWD (in meter) was paralleled by an improvement of asthma symptoms (as measured with ACT), quality of life (as measured with AQLQ), and Oxygen saturation. There is also a significant difference for the BORG Dyspnea Scale before treatment and a trend toward significance after treatment (p value=0.079) for the patients treated with mepolizumab. By paired comparisons, however, Δ (Δ= after – before mepolizumab) Borg Scale, as well as Δheart rate, Δsystolic and diastolic blood pressions did not significantly change, whereas only Δ6MWD and Δ%Oxygen saturation in ambient air did. Borg Dyspnea scale, a validated instrument used to measure self-reported dyspnea during submaximal exercise routinely administered during 6MWT, is likely not a sensitive tool to measure change over time for breathlessness (as compared to visual activity score (VAS) for instance, which has been shown to be also more reproducible), and reflecting more a general fatigue status than breathlessness(35).

The lack of physical activity in severe asthmatic patients has been shown in a recent study form Mancuso et al., reporting the lack of exercise due to barriers such as lack of motivation, time constraints, and extreme weather conditions, although most patients understood the importance of physical activity for their health status(22).

In addition, patients with more severe asthma were more likely to think that exercise was not beneficial for asthma. Therefore, the lack of exercise worsened feelings of anxiety and depression.

In addition, lower levels of sedentary time combined with higher levels of activity are associated with better asthma control, reducing oxidative stress, and improving quality of life(30).

This study has strengths and limitations. The strength is that these results are unique, since similar studies on 6MWT and asthma assessment after biologics lack in the field.

A limitation is the number of patients enrolled, which is small and limited to severe asthma in Italian patients, therefore these results are not generalizable to patients with less severe asthma or different ethnicities. In addition, we did not perform 6MWT between baseline and 12 months, therefore we cannot show how quick the improvement in 6MWD was, or if different pattern exists. In addition, this study is a pilot study and therefore further confirmations on larger populations are needed in the future.

CONCLUSIONS

Our study analyzed the possible relationship between severe eosinophilic asthma and 6MWT. Indeed, it shows a and the potential of 6MWT to complement severe asthma assessment, showing sensitivity to change after treatment, and good correlations with asthma parameters, such as symptom score, asthma-related quality of life score, and small airway dysfunction. Indirectly, the present study demonstrates that severe asthma patients increased their 6MWD after initiation of therapy (both biologic therapy with mepolizumab and conventional therapy), and this finding is in line with previous sparse studies performed about this topic(24, 29, 31, 32). As expected, the mepolizumab group had a major benefit from the treatment as compared to controls, especially at a pulmonary function level.

In view of these findings 6MWT could be considered a relevant and practical tool in the evaluation of the objective response of severe asthma treatment. Considering that 6MWT is also a simple and inexpensive test that evaluates physical performance in a standardized way, it could be useful to extend the 6MWT use in severe asthmatics to longitudinally monitor in a more objective fashion the impact of biologic therapies on asthma.

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DATA AVAILABILITY STATEMENT: All datasets generated for this study are included in the article.

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Table I: **Baseline characteristics of the 22 patients with severe asthma included in the study, 13 patients in the mepolizumab group (treated with mepolizumab) and 9 in the conventional therapy group (not treated with biological therapy). Data are presented mean±SD, as the mean±SD, median and n (%).**

Characteristics	Mepolizumab group	Conventional therapy group	P value
Clinical and laboratory features			
Age at asthma diagnosis, years, n (%)	35.2±11.3	36.4±9.6	0.645
Age at study enrollment, years, n (%)	53.0±10.4	54.1±8.9	0.651
Sex, female, n (%)	4 (44%)	8 (62%)	0.666
Former smokers, n (%)	4 (44%)	6 (46%)	1.000
BMI (kg/m^2), mean±SD	25.5±3.3	26.1±4.2	0.726
Nasal polyposis, n (%)	3 (23%)	3 (33%)	0.655
GERD, n (%)	3 (23%)	3 (23%)	1.000
Bronchiectasis, n (%)	4 (31 %)	3 (33 %)	1.000
Eosinophils in blood (Eos/mmc), mean±SD	548.7±216.9	466.4±197.1	0.375
FeNO50 (ppb), mean±SD	33.2±9.8	39.4±10.2	0.166
Pulmonary function test			
FEV1, % predicted, mean±SD	75.6±7.4	75.4±6.9	0.961
FVC, % predicted, mean±SD	101.1±2.2	100.6±2.3	0.641
FEF25-75%, % predicted, mean±SD	51.4±17.6	62.4±14.6	0.138
Asthma and quality of life scores			
ACT score, mean±SD	13.9±3.7	14.6±2.4	0.654
AQLQ score, mean±SD	4.3±1.0	4.1±0.8	0.661
Asthma treatments			
ICS (high dose)/LABA/LAMA, n (%)	12 (92%)	7 (78%)	0.5442
ICS (low/moderate dose)/LABA, n (%)	1 (8%)	2 (22%)	0.5442
Continuous OCS use, n (%)	6 (46%)	4 (44%)	1.000

Legenda: FEV1: forced expiratory volume in 1s; FVC: forced vital capacity; FEF: mid-expiratory flow rate, ACT: asthma control test; AQLQ: asthma quality of life questionnaire; FeNO: fractional exhaled nitric oxide; GERD: gastroesophageal reflux disease; BMI: body mass index, ICS: inhaled corticosteroids; LABA: long acting beta2 agonist; LAMA: long acting muscarinic antagonist; OCS: oral corticosteroids.

Table II: **The pulmonary function, BMI, clinical, laboratory and treatment features at baseline (T0) and at 1 year (T1). The Mepolizumab group is treated with mepolizumab 100mg/month for 12 month-, while the conventional therapy group is not treated with biological therapy for asthma Data are presented as the mean±SD and n (%).**

Characteristics	Mepolizumab group			Conventional therapy group		
	Baseline (T0)	At 1 year (T1)	P value (95% CI)	Baseline (T0)	At 1 year (T1)	P value (95% CI)
Pulmonary function test and BMI						
FEV1, % predicted, mean±SD	75.6±7.4	84.1±5.8	<0.001 (4.6;12.4)	75.4±6.9	80.8±6.7	0.113 (-1.4; 12.1)
FVC, % predicted, mean±SD	101.1±2.2	105.2±3.4	0.002 (1.8;6.5)	100.6±2.3	101.8±2.5	0.292 (-1.2; 3.6)
FEF25-75%, % predicted, mean±SD	51.4±17.6	65.37±14.4	<0.001 (8.5;19.5)	62.4±14.6	68.4±13.3	0.376 (-8.0; 19.9)
BMI (kg/m^2)	25.5±3.3	24.2±2.8	0.260 (-3,8;1.1)	26.1±4.2	27.3±3.8	0.544 (-3.1; 5.6)
Asthma and quality of life scores						
ACT score, mean±SD	13.9±3.7	21.5±2.1	<0.001 (5.0;10.2)	14.6±2.4	21.3±1.1	<0.001 (4.6;8.8)
AQLQ score, mean±SD	4.3±1.0	5.5±0.7	0.001 (0.6;1.8)	4.1±0.8	5.4±0.6	0.005 (0.4;2.1)
Laboratory features						
Eosinophils in blood (Eos/mmc), mean±SD	548.7±216.9	109.3±41.1	<0.001 (-555.8;-322.9)	466.4±197.1	424.6±125.0	0.633 (-225.6;141.8)
FeNO50 (ppb), mean±SD	33.2±9.8	23.5±8.6	0.008 (-16.4;-2.9)	39.4±10.2	23.3±6.5	0.003 (-25.7;-6.7)
Asthma treatments						
ICS (high dose)/LABA/LAMA, n (%)	12 (92%)	11 (85%)	1.000	7 (78%)	2 (22%)	1.000
ICS (low/moderate dose)/LABA, n (%)	1 (8%)	2 (13%)	1.000	2 (22%)	1 (11%)	1.000
Continuous OCS use , n (%)	6 (46%)	3 (23%)	0.248	4 (44%)	5 (56%)	1.000

Legenda: FEV1: forced expiratory volume in 1s; FVC: forced vital capacity; FEF: mid-expiratory flow rate, ACT: asthma control test; AQLQ: asthma quality of life questionnaire; FeNO: fractional exhaled nitric oxide; GERD: gastroesophageal reflux disease; BMI: body mass index; ICS: inhaled corticosteroids; LABA: long acting beta2 agonist; LAMA: long acting muscarinic antagonist; OCS: oral corticosteroids.

Table III: The 6-minute walking test (6MWT) data and other cardiovascular parameters at baseline (T0) and at 1 year (T1). The Mepolizumab group is treated with mepolizumab 100mg/month for 12 month, while the conventional therapy group is not treated with biological therapy for asthma. Data are presented as the mean ± SD and n (%).

6MWT and other cardiovascular parameters	Mepolizumab group			Conventional therapy group		
	Baseline (T0)	At 1 year (T1)	P value (95% CI)	Baseline (T0)	At 1 year (T1)	P value (95% CI)
Walking distance (m)	438.2±46.2	506.8±57.7	<0.001 (53.6;83.8)	450.3±27.5	506.0±41.6	0.004 (20.4;90.9)
Dyspnea Borg Scale						
baseline	1.9± 1.4 Median=2	0.8±1.5 Median=0	0.002 (-1.5;-1.0)	1.6±0.5 Median=2	0.8±0.7 Median=1	0.014 (-1.4;-0.2)
end	3.3±1.3 Median=3	2.3±2.1 Median=1	0.079 (-3;0.5)	4.1±0.8 Median=4	2.4±1.0 Median=2	0.001 (-2.6;-0.8)
Heart Rate (HR/min)						
baseline	78.8±12.8	74.8±9.0	0.025 (-7.5;-0.6)	73.8±13.0	75.9±6.2	0.665 (-8.0;12.2)
End	102.5±14.8	98.5±10.3	0.159 (-9.8;1.8)	102.3±8.0	100.1±7.1	0.666 (-9.1;6.0)
Systolic Blood Pressure						
Baseline	124.6±11.9	123.1±9.3	0.717 (-10,2;7.1)	125.6±11.8	122.8±6.2	0.542 (-12.2;6.7)
End	126.9±10.5	127.3±8.8	0.765 (-1.6;2.3)	142.8±12.5	141.7±7.9	0.825 (-11.6;9.4)
Dyastolic Blood Pressure						
Baseline	76.1±6.2	75.4±4.8	0.738 (-5.7;4.1)	82.8±5.1	80.0±3.5	0.196 (-7.1;1.6)
End	76.1±7.7	77.3±4.4	0.641 (-4.1;6.4)	87.2±6.7	85.6±7.3	0.619 (-8.6;5.3)
SpO2 (%)						
Baseline	95.8±1.2	97.1±0.6	0.0001 (0.6;1.8)	95.6±0.9	96.8±0.7	0.004 (0.4;2.0)
End	95.5±0.9	97.1±0.8	0.0001 (1.0; 2.2)	95.0±0.9	95.9±1.6	0.165 (-0.4;2.2)

Mean	95.0±1.2	96.0±1.1	0.006 (0.9;1.5)	95.0±0.7	95.8±1.1	0.092 (-0.1;1.7)

Legenda: SpO2 : pulsed oxygen saturation; m : meter; 6MWT: 6 min walk test.

FIGURE LEGENDS

Figure 1. 6-minute walking test (6MWT) at baseline (T0) and after 1 year of observation (T1). Paired comparisons in the mepolizumab group (before and after mepolizumab, panel A) and in the conventional therapy group (panel B) of the 6-minute walking test (6MWT) data and other cardiovascular parameters. Significant p value was reported as * when <0.05, ** when <0.01, * when <0.001.**

Figure 2. - Individual 6-minute walking distance (6MWD) in the studied patients (Figure 2A). Red line refers to the minimal important difference (MID, 25 meters), conventionally used as a cut-off. Correlations between 6MWD pulling together T0 and T1 of the 22 patients studied (n=44) and ACT score (Figure 2B), AQLQ score (Figure 2C), and FEF25-75% (Figure 2D). Correlations are calculated with the Spearman's method and reported in the figure as r and p value. 95% CI and details can be found in Supplementary Table I.

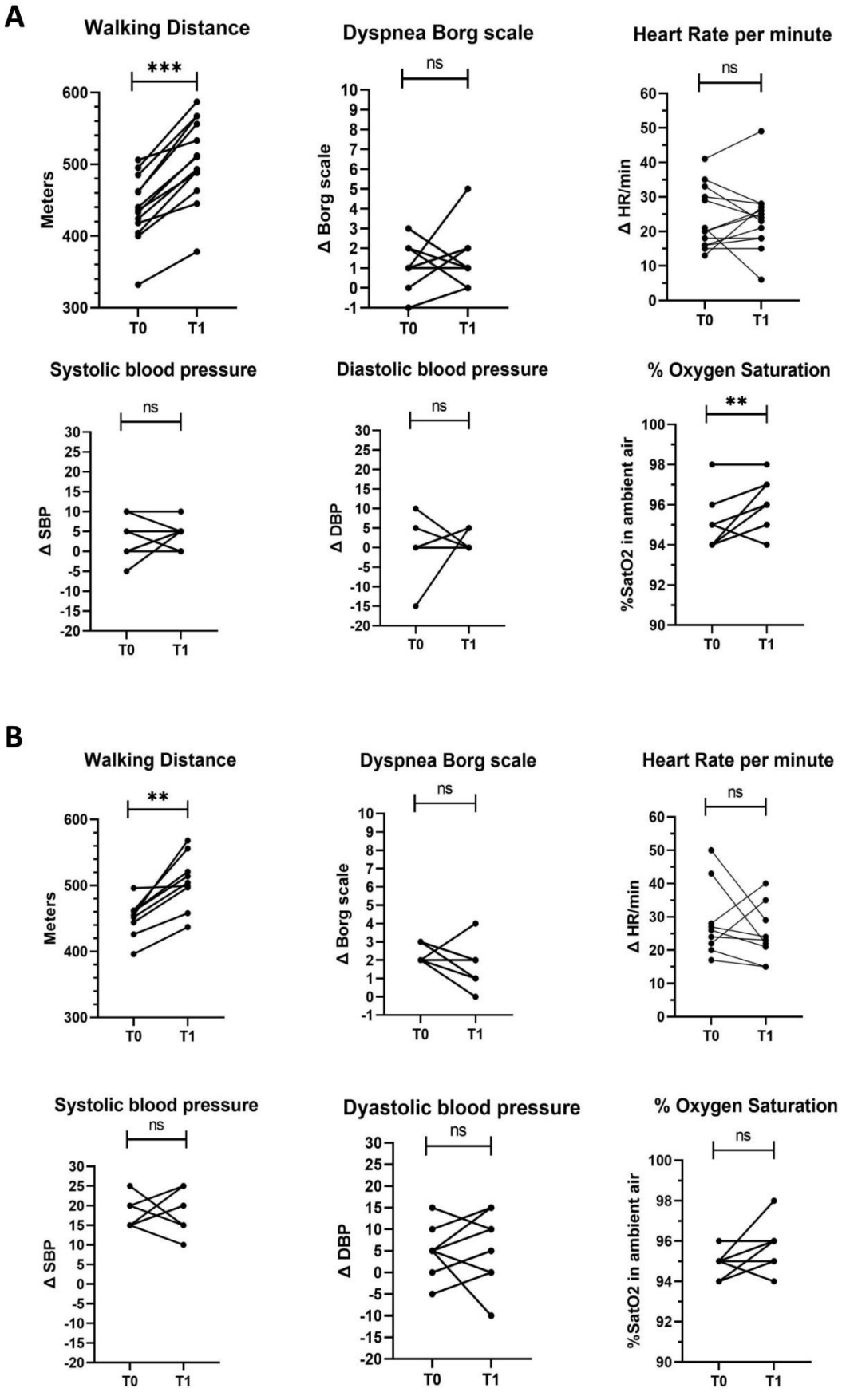
A
Walking Distance
***
Meters
600
500
400
300
T0
T1
Dyspnea Borg scale
ns
Δ Borg scale
10
9
8
7
6
5
4
3
2
1
0
-1
T0
T1
Heart Rate per minute
ns
Δ HR/min
60
50
40
30
20
10
0
T0
T1
Systolic blood pressure
ns
Δ SBP
30
25
20
15
10
5
0
-5
-10
-15
-20
T0
T1
Diastolic blood pressure
ns
Δ DBP
30
25
20
15
10
5
0
-5
-10
-15
-20
T0
T1
% Oxygen Saturation
**
%SatO2 in ambient air
100
98
96
94
92
90
T0
T1
B
Walking Distance
**
Meters
600
500
400
300
T0
T1
Dyspnea Borg scale
ns
Δ Borg scale
10
9
8
7
6
5
4
3
2
1
0
-1
T0
T1
Heart Rate per minute
ns
Δ HR/min
60
50
40
30
20
10
0
T0
T1
Systolic blood pressure
ns
Δ SBP
30
25
20
15
10
5
0
-5
-10
-15
-20
T0
T1
Dyastolic blood pressure
ns
Δ DBP
30
25
20
15
10
5
0
-5
-10
-15
-20
T0
T1
% Oxygen Saturation
ns
%SatO2 in ambient air
100
98
96
94
92
90
T0
T1

Figure 1

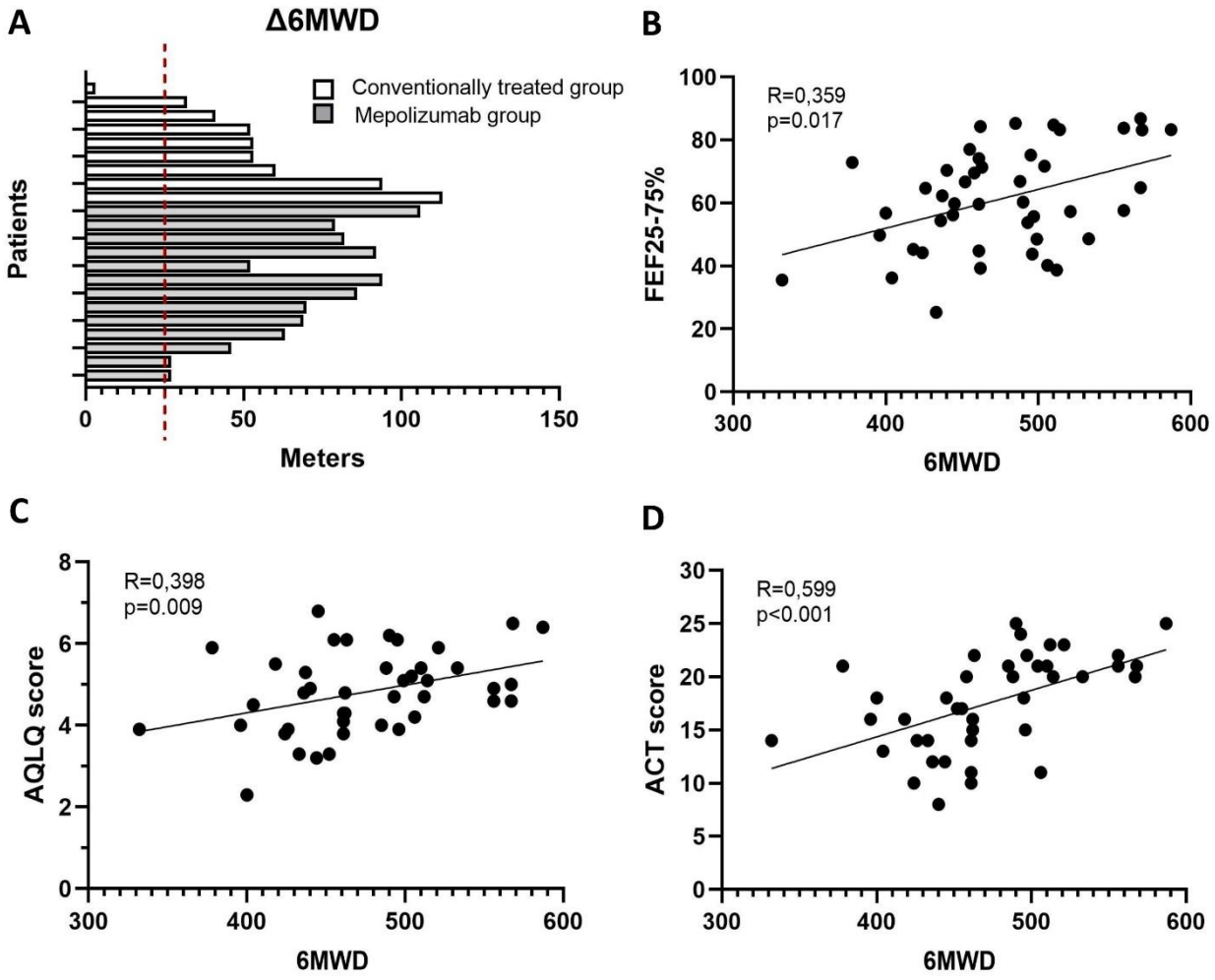
A
Δ6MWD
Conventionally treated group
Mepolizumab group
Patients
0
50
100
150
Meters
B
R=0,359
p=0.017
FEF25-75%
100
80
60
40
20
0
300
400
500
600
6MWD
C
R=0,398
p=0.009
AQLQ score
8
6
4
2
0
300
400
500
600
6MWD
D
R=0,599
p<0.001
ACT score
30
25
20
15
10
5
0
300
400
500
600
6MWD

Figure 2

SUPPLEMENTARY MATERIALS

Supplementary Table I. Correlations between 6-minute walking test (6MWT), asthma symptoms (Asthma Control Test, ACT), asthma-related quality of life (Asthma Quality of Life Questionnaire, AQLQ), and functional parameters (FEV1%, FVC%, FEV1/FVC%, FEF25-75%) in the 22 patients with severe asthma. The correlations are calculated between Δ6MTD (T1-T0) and ΔACT, ΔAQLQ and Δfunctional parameters (T1-T0) in the 22 patients with severe asthma, or between the values of 6MWD and ACT, ACQ and functional parameters pulling together T0 and T1 (in total n=44). Correlations are calculated with the Spearman's method. In the table is reported r (95%CI). Correlation with a p value < 0,05 are reported in bold.

	ΔACT		**ΔAQLQ**		**ΔFEV1%**		**ΔFVC%**		**ΔFEV1/FVC%**		**ΔFEF25-75%**	
	r	**(95%CI)**	**r**	**(95%CI)**	**r**	**(95%CI)**	**r**	**(95%CI)**	**r**	**(95%CI)**	**r**	**(95%CI)**
Δ6MWD (n=22)	0.2044	-0.2502 to 0.5852	-0.2984	-0.6473 to 0.1540	-0.2593	-0.6220 to 0.1651	0.0964	-0.3507 to 0.5077	-0.1956	-0.5791 to 0.258	-0.0221	-0.4503 to 0.4144
	ACT		**AQLQ**		**FEV1%**		**FVC%**		**FEV1/FVC%**		**FEF25-75%**	
	r	**(95%CI)**	**r**	**(95%CI)**	**r**	**(95%CI)**	**r**	**(95%CI)**	**r**	**(95%CI)**	**r**	**(95%CI)**
Pulled 6MWD (n=44)	**0.5998**	**0.3537 to 0.7682**	**0.3978**	**0.0976 to 0.6317**	0.2650	-0.0437 to 0.5274	0.1760	-0.1364 to 0.4566	0.2222	-0.0889 to 0.1938	**0.3589**	**0.0605 to 0.5985**