Using the 6-min walk test to assess the clinical response to mepolizumab and conventional therapy in severe eosinophilic asthma

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Shareable abstract (@ERSpublications)
6-min walk distance shows sensitivity to change, and correlates with asthma symptoms, quality of life and small airway dysfunction. Thus, it could be relevant for evaluating the objective response to severe asthma treatments. https://bit.ly/3CRwndi

Cite this article as: Lombardi C, Berti A, Cottini M, *et al*. Using the 6-min walk test to assess the clinical response to mepolizumab and conventional therapy in severe eosinophilic asthma. *ERJ Open Res* 2023; 9: 00114-2023 [DOI: 10.1183/23120541.00114-2023].

Received: 26 Feb 2022
Accepted: 21 June 2023

Abstract

Background Severe asthma limits exercise to avoid respiratory symptoms. The objective of the present study was to investigate the role of the 6-min walk test (6MWT) in severe asthma.

Methods Consecutive patients with severe eosinophilic asthma were enrolled. A 6MWT was performed before and after 12 months. Inhaled therapy dose, oral corticosteroids dose, pulmonary function tests, eosinophil blood count, fractional exhaled nitric oxide (F_{eNO}), Asthma Control Test (ACT) score and responses to the Asthma Quality of Life Questionnaire (AQLQ) were also recorded.

Results Of the 22 patients enrolled, 13 were treated with mepolizumab 100 mg every 4 weeks in addition to conventional therapy and nine with conventional therapy only. The majority of the patients were treated with high-dose inhaled corticosteroids/long-acting β-agonists/long-acting muscarinic receptor antagonists, while approximately half were on continuous oral corticosteroids. After 12 months, the mepolizumab group only showed a significant improvement in pulmonary function tests (percentage forced expiratory volume in 1 s and percentage forced expiratory flow at 25–75% forced vital capacity ($FEF_{25–75\%}$), both p<0.001; percentage forced vital capacity, p<0.01) and clinical laboratory parameters (eosinophil count, F_{eNO} measured at a flow rate of 50 mL·s^{-1}, ACT and AQLQ, p<0.001). No significant changes in the proportion of patients using continuous oral corticosteroids and high-dose inhaled corticosteroids/long-acting β-agonists/long-acting muscarinic receptor antagonists were observed in either group (p>0.05). By paired comparisons, statistically significant improvements of the mean 6-min walk distance (6MWD) were observed in the mepolizumab (p<0.001) and conventional therapy (p<0.01) groups, while no improvement was seen in dyspnoea Borg scale, heart rate, percentage oxygen saturation or systolic and diastolic blood pressure. 6MWD showed significant direct correlations with ACT (r=0.5998, p<0.001), AQLQ (r=0.3978, p=0.009) and $FEF_{25–75\%}$ (r=0.3589, p=0.017).

Conclusions The 6MWT could complement severe asthma assessment and be relevant in evaluating the objective response to treatment, including biological therapies like mepolizumab.

Introduction

The 6-min walk 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 (ATS) and officially introduced in 2002, along with a comprehensive guideline [4]. The test was initially designed to help in the assessment of patients with cardiopulmonary issues. It was gradually introduced in other conditions, such as asthma and chronic obstructive pulmonary disease (COPD). The 6MWT evaluates functional

capacity and 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 who otherwise have difficulty in performing standard exercise tests, and the 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. Dajczman *et al*. [11] demonstrated that overall survival at 3 years was only 58% and was especially poor (34%) in COPD patients with low (<150 m) initial walk distance. An increase in the distance walked indicates improvement in basic mobility. At least in patients with severe COPD, the most recently updated minimal important difference in exercise tests is about 25 m, which means that in amputee rehabilitation post training a difference of at least 45 m should be observed for the 6MWT to ensure a reasonably substantial change [12]. However, the 6MWT cannot give a pathophysiological insight into the exercise intolerance/dyspnoea of patients, unlike other tests such as cardiopulmonary exercise testing, which is more time-consuming and exposes the patients to some risks from a cardiovascular standpoint.

In contrast to other diseases studied using the 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 of patients and their families. The magnitude of this morbidity is affected by several personal factors, including age. The use of biological therapies has shown some promising effects on the quality of life of patients with severe uncontrolled asthma [15].

Moreover, patients with severe asthma have some functional similarities to 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 biological agents in severe asthma.

In this study, we performed a prospective analysis of the 6MWT in patients 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 interleukin-5, while comparing these findings to a 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 parameters with specific asthma scores and functional features.

Materials and methods

Study setting and participants

This was a single-centre study conducted in an accredited outpatient Severe Asthma Clinic (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)/ATS criteria [13] were prospectively enrolled. Owing to the COVID-19 pandemic, after the first patients were enrolled the service was temporarily suspended. Even after the first two waves of the COVID-19 pandemic, the 6MWT was not regularly performed in Poliambulanza due to safety reasons. Initially, 50 patients were evaluated in the pre-screening visit, but only three patients were enrolled and completed the two 6MWTs 12±1 months apart until March 2021; therefore, most of the patients in 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), who initially evaluated the patient before enrolment (screening visit) and after 4 weeks (visit T0). Before the screening visit and visit T0, the treating physician optimised inhaled therapy and checked comorbidities and treatment compliance. Therefore, each patient completed at least 4 weeks of asthma therapy after a structured medical assessment. The first 6MWT was done at visit T0.

The initial patient assessment was performed with standard investigations such as family history, personal history, pathological medical history, pulmonary function tests, eosinophil count, fractional exhaled nitric oxide (F_{eNO}), skin prick tests, serum-specific IgE and high-resolution computed 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) was used to evaluate disease-specific health-related quality of life (scores range from 1 to 7, with higher scores indicating better quality of life). Patients were also assessed for the presence of nasal polyposis and/or gastro-oesophageal reflux.

To avoid the confounding effect of smoke, current smokers were excluded from this study, as well as patients engaged in exercise training programmes 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 were patients fulfilling the criteria for asthma–COPD overlap syndrome.

We compared these results with those from consecutive patients with severe eosinophilic asthma who were not treated with biological therapy (data for this cohort were collected before Agenzia Italiana del Farmaco (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 a 6MWT performed at T0 and T1 were included, as per the study cohort, and were evaluated with the same structured clinical assessment and asthma and quality of life scores. The number of patients in the control group was 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 performed a 6MWT in the days immediately preceding the start of observation (and the start of biological treatment in the mepolizumab group) (T0) and 12 months after starting observation (T1), along with a physical examination, blood eosinophil count, spirometry, F_{eNO}, ACT and AQLQ completion.

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

A total of 13 patients had been treated with subcutaneous mepolizumab 100 mg every 4 weeks in addition to conventional nonbiological therapies for asthma, while nine patients were treated with conventional therapies only.

Information on 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 the Global Initiative for Asthma guidelines [16].

6MWT

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

Pulse oximeter specifications were as follows: peripheral oxygen saturation (S_{pO_2}) measurement: 0–99% (S_{pO_2} accuracy: ±2% between 70–99% S_{pO_2}); pulse rate measurement: 30–254 beats·min^{-1} (pulse rate accuracy: ±2 beats·min^{-1} or 2%, whichever is greater).

Pulse oximeter measured parameters were as follows: S_{pO_2} (basal, minimum, maximum, mean), pulse rate (basal, minimum, maximum, mean), T90 (S_{pO_2}<90%), T89 (S_{pO_2}<89%), T88 (S_{pO_2}<88%), T5 (ΔS_{pO_2}>5%), Δindex (12 s), S_{pO_2} events (pulse rate events, bradycardia, tachycardia), number of steps and movement (vector magnitude units).

The 6MWT parameters collected were O_2 gap, estimated distance, distance walked, theoretical distance (minimum, standard), TΔ2 (S_{pO_2} ⩾2%), TΔ4 (ΔS_{pO_2} ⩾4%), recording time, time (rest, walk, recovery), desaturation area/distance, Borg dyspnoea (start, end, difference), Borg fatigue (start, end, difference), blood pressure (systolic, diastolic) and oxygen administered.

The test was conducted according to the ERS/ATS international standard guidelines [17].

Modified Borg dyspnoea scale

The ATS 2002 guidelines suggest the modified Borg scale as an aid for the 6MWT, enabling the degree of respiratory discomfort to be evaluated in terms of determination 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 on the purpose of the scale and 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 dyspnoea.

Statistical analysis

Data are summarised using percentages, mean±SD or median (IQR 25–75%). Variations in time of the quantitative characteristics, 6MWT and other cardiovascular parameters were assessed using a t-test or a nonparametric 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 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 analyse paired data before and after the observation time. Spearman's method was used to test for possible correlations between the studied variables.

Data were analysed using the statistical software R (www.r-project.com) and GraphPad Prism (San Diego, CA, USA).

Results

We included 22 patients with severe eosinophilic asthma who performed a 6MWT and completed functional and clinical respiratory assessment at baseline and after 1 year of observation; 13 patients in the group were treated with mepolizumab and nine patients were treated only with conventional therapies (*i.e.* not treated with biological therapy). There were no differences at baseline between cases and controls in demographics, clinical features, laboratory or pulmonary function tests or asthma treatment before the observation period (table 1).

In the mepolizumab group, the mean age at enrolment was 53.0±10.4 years, while the age at asthma diagnosis was 35.2±11.3 years. Body mass index (BMI) at enrolment was 27.9±7.1 $kg \cdot m^{-2}$. Mean eosinophil count at enrolment was 548.7±216.9 $cells \cdot mm^{-3}$, 23% of patients had nasal polyposis, 23% had gastrointestinal reflux disease and 31% had bronchiectasis. 92% of patients were treated with high-dose inhaled corticosteroid (ICS)/long-acting β-agonist (LABA)/long-acting muscarinic receptor antagonist (LAMA), while 46% were on continuous OCS. All 13 patients in the mepolizumab group started treatment with mepolizumab 100 mg every 4 weeks at enrolment, fulfilling the AIFA prescribing criteria.

After 1 year of observation, in the mepolizumab group there was a significant mean improvement in pulmonary function test results (% forced expiratory volume in 1 s (FEV_1), 95% CI of the mean difference 4.6–12.4%, $p<0.001$; % forced expiratory flow at 25–75% ($FEF_{25–75\%}$), 95% CI 8.5%–19.5%, $p<0.001$; % forced vital capacity (FVC), 95% CI 1.8%–6.5%, $p<0.01$), asthma and quality of life questionnaire scores (ACT score, 95% CI 5.0–10.2, $p<0.001$; AQLQ score, 95% CI 0.6–1.8, $p<0.001$), blood eosinophils (95% CI −555.8– −322.9 $cells \cdot mm^{-3}$, $p<0.001$) and $F_{eNO_{50}}$ (95% CI −16.4– −2.9 ppb, $p<0.001$). In contrast, in the conventional therapy group there was a mean significant improvement only in the ACT score, AQLQ score and F_{eNO}, without pulmonary function test or eosinophil level modifications (table 2).

No significant change in the proportion of patients using continuous OCS or inhaled therapy (ICS/LABA/LAMA) or in BMI were observed in either group ($p>0.05$) (table 2). None of the patients had at baseline or developed between T0 and T1 new cardiovascular or musculoskeletal issues that could have impaired

TABLE 1 Baseline characteristics of the 22 patients with severe asthma included in the study

Characteristics	Mepolizumab group	Conventional therapy group	p-value
Patients (n)	13	9	
Clinical and laboratory features			
Age at asthma diagnosis (years)	35.2±11.3	36.4±9.6	0.645
Age at study enrolment (years)	53.0±10.4	54.1±8.9	0.651
Female sex	4 (44)	8 (62)	0.666
Former smoker	4 (44)	6 (46)	1.000
BMI ($kg \cdot m^{-2}$)	25.5±3.3	26.1±4.2	0.726
Nasal polyposis	3 (23)	3 (33)	0.655
GORD	3 (23)	3 (23)	1.000
Bronchiectasis	4 (31)	3 (33)	1.000
Eosinophils in blood ($cells \cdot mm^{-3}$)	548.7±216.9	466.4±197.1	0.375
$F_{eNO_{50}}$ (ppb)	33.2±9.8	39.4±10.2	0.166
Pulmonary function tests			
FEV_1 % predicted	75.6±7.4	75.4±6.9	0.961
FVC % predicted	101.1±2.2	100.6±2.3	0.641
$FEF_{25–75\%}$ % predicted	51.4±17.6	62.4±14.6	0.138
Asthma and quality of life scores			
ACT score	13.9±3.7	14.6±2.4	0.654
AQLQ score	4.3±1.0	4.1±0.8	0.661
Asthma treatments			
ICS (high dose)/LABA/LAMA	12 (92)	7 (78)	0.5442
ICS (low/moderate dose)/LABA	1 (8)	2 (22)	0.5442
Continuous OCS use	6 (46)	4 (44)	1.000

Data are presented as mean±SD and n (%), unless otherwise stated. BMI: body mass index; GORD: gastro-oesophageal reflux disease; $F_{eNO_{50}}$: fractional exhaled nitric oxide measured at a flow rate of $50\ mL \cdot s^{-1}$; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of the forced vital capacity; ACT: Asthma Control Test; AQLQ: Asthma Quality of Life Questionnaire; ICS: inhaled corticosteroid; LABA: long-acting β2 agonist; LAMA: long-acting muscarinic antagonist; OCS: oral corticosteroid.

the results of the 6MWT, and patients denied any modification in physical habits during the observation period.

When focusing on mean 6MWT parameters (table 3), in the mepolizumab group there were statistically significant improvements in the 6MWD (from 438.2±46.2 m to 506.8±57.7 m, p<0.001) and mean % S_{pO_2} (from 95.0±1.2% to 96.0±1.1%, p=0.006). In the conventional therapy group, there was improvement in the mean 6MWD (from 450.3±27.5 m to 506.0±41.6 m, p=0.004) but no significant change in mean % S_{pO_2}. There were some improvements in the mean values of the Borg dyspnoea scale in both groups at T1 as compared to T0, while there were no statistically significant changes for heart rate or systolic and diastolic blood pressure in either group.

By paired comparisons, the 6MWD showed 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 S_{pO_2} accuracy of the instrument was greater than the mean difference measured (±2%), 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 patients; in 11 out of 13 patients this improvement was >45 m, and in 13 out of the 13 patients it was >25 m, two recognised cut-offs used for COPD (figure 2a), with an overall mean 6MWD of 69±25 m (range: 27–106 m). In the conventional therapy group, in six out of nine patients the 6MWD was >45 m, and in eight out of nine patients it was >25 m, with an overall mean 6MWD of 56±32 m (range: 3–113 m; p=0.594 compared with mepolizumab group).

No correlations were observed between the changes in 6MWD (value at T1–value at T0), asthma symptom score (as assessed by ACT), asthma-related quality of life score (as assessed by AQLQ) or functional parameters (FEV_1 %, FVC %, FEV_1/FVC %, $FEF_{25–75\%}$) (supplementary table S1). However, when the crude 6MWD values were pulled together (T0+T1, n=44) and correlated with the crude values of the same

TABLE 2 Pulmonary function, BMI, clinical, laboratory and treatment features at baseline and at 1 year

Characteristics	Mepolizumab group[#]			Conventional therapy group[¶]		
	Baseline (T0)	1 year (T1)	p-value (95% CI)	Baseline (T0)	1 year (T1)	p-value (95% CI)
Pulmonary function test and BMI						
FEV_1 % predicted	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	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)
$FEF_{25–75\%}$ % predicted	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	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	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 ($cells·mm^{-3}$)	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)
$F_{eNO_{50}}$ (ppb)	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	12 (92)	11 (85)	1.000	7 (78)	2 (22)	1.000
ICS (low/moderate dose)/LABA	1 (8)	2 (13)	1.000	2 (22)	1 (11)	1.000
Continuous OCS use	6 (46)	3 (23)	0.248	4 (44)	5 (56)	1.000

Data are presented as mean±SD or n (%), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of the forced vital capacity; ACT: Asthma Control Test; AQLQ: Asthma Quality of Life Questionnaire; $F_{eNO_{50}}$: fractional exhaled nitric oxide measured at a flow rate of 50 $mL·s^{-1}$; ICS: inhaled corticosteroids; LABA: long-acting β2 agonist; LAMA: long-acting muscarinic antagonist; OCS: oral corticosteroids. [#]: the mepolizumab group was treated with mepolizumab 100 mg every 4 weeks for 12 months; [¶]: the conventional therapy group was not treated with a biological agent for asthma.

asthma and quality of life scores and functional parameters, significant, direct correlations were observed between 6MWD and ACT, AQLQ and $FEF_{25–75\%}$ (supplementary table S1, figure 2b–d).

Discussion

We have shown that in patients with severe asthma, 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 by $FEF_{25–75\%}$). In addition, 6MWD improved after 12 months of observation in our relatively small but very well characterised cohort of 22 patients with severe asthma (13 patients treated with mepolizumab, nine with conventional therapy), in contrast with the other cardiorespiratory parameters measured during the 6MWT. Notably, the improvement was observed in 100% of the patients treated with mepolizumab, and in all the improvement was clinically significant (*i.e.* >25 m) according to the published cut-off used for COPD [12]. The mepolizumab group received 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, F_{eNO}, ACT and AQLQ, indicating that the 6MWD improvement was paralleled by a functional and disease-related biomarker improvement, along with the improvement of quality of life. Taken altogether, our data proved the concept that the 6MWT can be relevant for objectively evaluating treatment response in severe asthma.

Exercise capacity is associated with symptoms and health-related quality of life in severe asthma. Indeed, dyspnoea on exertion is the most reported symptom described by patients with uncontrolled asthma, limiting everyday life activity [19–21]. 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].

TABLE 3 The 6-min walk test data and other cardiovascular parameters at baseline and at 1 year

Parameters	Mepolizumab group[#]			Conventional therapy group[¶]		
	Baseline (T0)	1 year (T1)	p-value (95% CI)	Baseline (T0)	1 year (T1)	p-value (95% CI)
6MWD (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)
Borg dyspnoea 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 (beats·min^{-1})						
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)
SBP (mmHg)						
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)
DBP (mmHg)						
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)
S_{pO_2} (%)						
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)

Data are presented as mean±SD and n (%), unless otherwise stated. 6MWD: 6-min walk distance; SBP: systolic blood pressure; DBP: diastolic blood pressure; S_{pO_2}: peripheral oxygen saturation. [#]: the mepolizumab group was treated with mepolizumab 100 mg every 4 weeks for 12 months; [¶]: the conventional therapy group was not treated with a biological agent for asthma.

Some asthmatic patients report exercise intolerance leading to limitations in daily life activities as the most prominent symptom, rather than the wheezing attacks, severely affecting 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 rarely been evaluated, and 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 (assessed using FEV_1 and FEV_1/FVC) [23, 25–28].

Significantly, of the correlations among 6MWD and functional parameters, asthma symptoms and quality of life, only $FEF_{25–75\%}$, ACT (the strongest correlations) and AQLQ were significant. To us, this suggests that the relationship between the 6MWD could be linked to the function of small airways, which is linked to exercise-induced asthma [28, 29], correlating with asthma symptoms and quality of life. Given that the ultimate goal of asthma therapy is to achieve patient wellbeing, the 6MWD might be a way to objectively assess the "overall" status of these patients. From a treatment perspective, the advances in phenotyping and endotyping of severe asthma have given us new insights into personalised therapies, *i.e.* biological agents for severe asthma. Biological therapies target cytokines/mediators (*e.g.* interleukin-5), 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 affecting the natural course of asthma, *i.e.* reducing the exacerbation and avoiding airway remodelling. In addition, they usually allow a reduction in OCS and ICS doses, avoiding the short- and long-term adverse effects connected to their use and improving patient quality of life and socioeconomic costs [30].

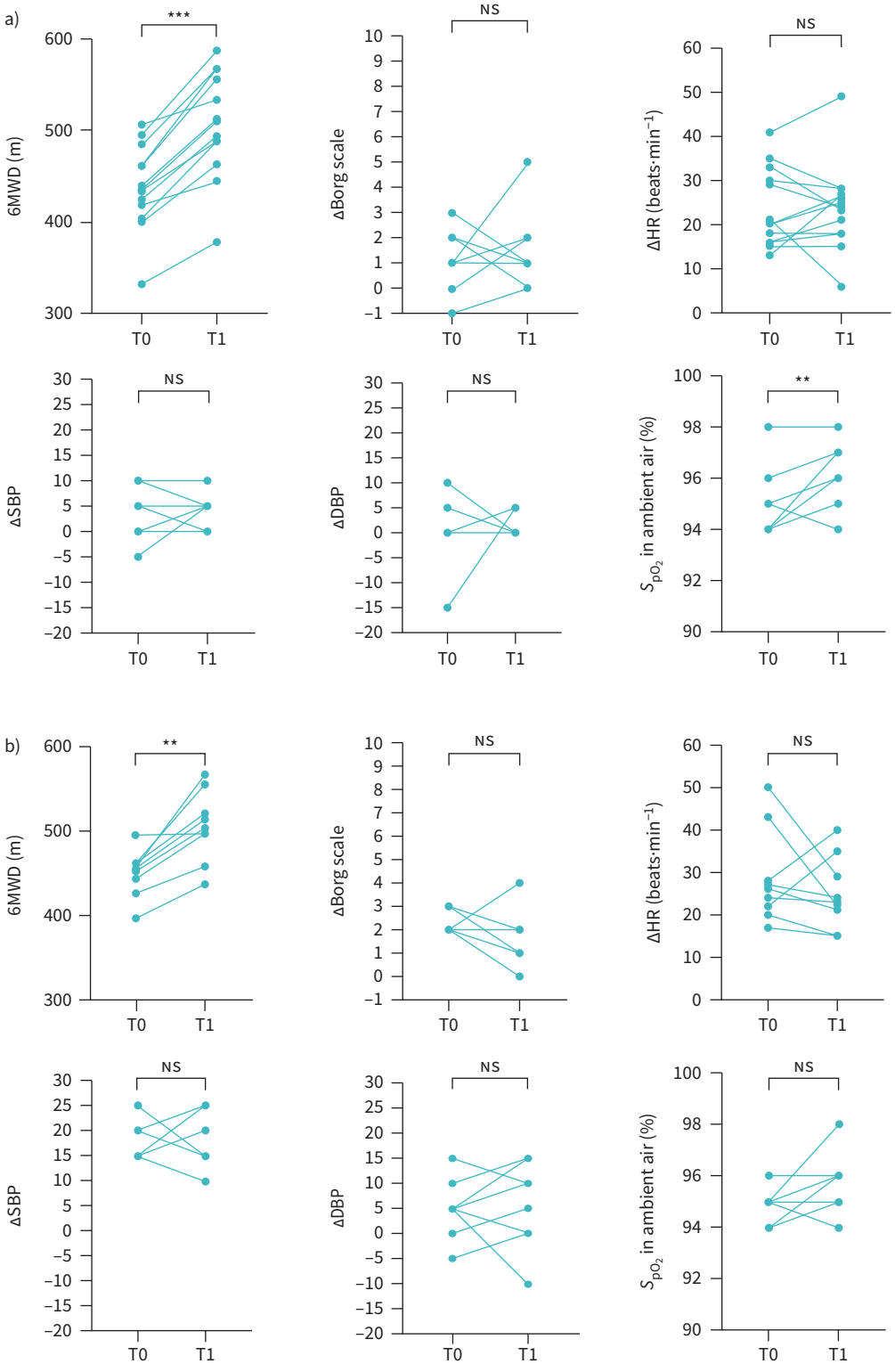

FIGURE 1 Paired comparisons in the a) mepolizumab group (before and after mepolizumab) and b) conventional therapy group of the 6-min walk distance (6MWD) data, Borg dyspnoea scale, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and peripheral oxygen saturation (S_{pO_2}) in ambient air at baseline (T0) and after 1 year of observation (T1). **: p<0.01; ***: p<0.001.

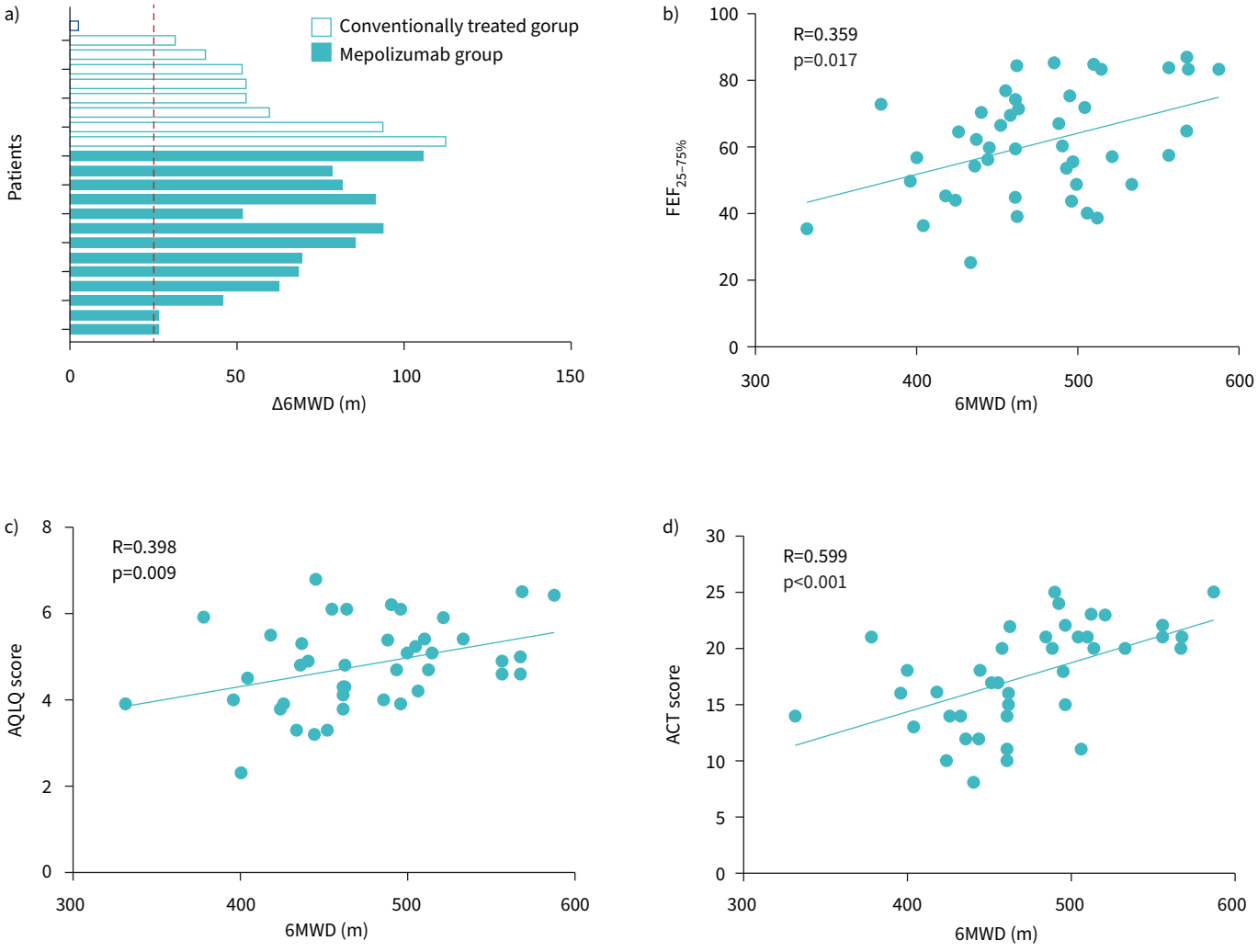

FIGURE 2 a) Individual 6-min walk distance (6MWD) in the studied patients. Red line refers to the minimal important difference (25 m) conventionally used as a cut-off. b–d) Correlations between 6MWD pulling together data from baseline and after 1 year of observation of the 22 patients studied (n=44) and forced expiratory volume at 25–75% of the forced vital capacity ($FEF_{25-75\%}$) (b), Asthma Quality of Life Questionnaire (AQLQ) score (c) and Asthma Control Test (ACT) score (d). Correlations were 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 S1.

Multiple randomised controlled trials on patients with severe eosinophilic asthma have demonstrated the efficacy of mepolizumab in reducing blood eosinophilia. This has been associated with a reduction in the rate of severe exacerbations and OCS use and improved asthma control and lung function [30]. In contrast, an improvement in exercise has never been investigated in the context of a clinical trial. In our cohort, the reduction in eosinophil count did not correlate with the change in 6MWD (not shown).

Carpagnano *et al.* [31] investigated physical activity in two groups of patients with severe asthma, analysing the changes occurring in 30 patients treated with biological therapies (omalizumab and mepolizumab) and 20 patients who underwent traditional treatment over 6 months. This study was a pioneer trial because it was the first to show a positive correlation between biological 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 a surrogate of clinical activity to measure the impact of mepolizumab in asthma. We found that the mean variation of 6MWD (in metres) was paralleled by an improvement in asthma symptoms (as measured with ACT), quality of life (as measured with AQLQ) and oxygen saturation. There was also a significant difference for

the Borg dyspnoea scale before treatment and a trend towards significance after treatment (p=0.079) for the patients treated with mepolizumab. By paired comparisons, however, the change in Borg scale (after–before mepolizumab), as well as change in heart rate and systolic and diastolic blood pressure, did not significantly alter, whereas change in 6MWD and % oxygen saturation in ambient air did. The Borg dyspnoea scale, a validated instrument used to measure self-reported dyspnoea 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 for instance, which has been shown to be also more reproducible) because it reflects more a general fatigue status than breathlessness [32].

Mancuso *et al*. [22] recently demonstrated a lack of physical activity in patients with severe asthma. They reported the lack of exercise to be 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. In addition, patients with more severe asthma are more likely to think that exercise is not beneficial for asthma. Therefore, the lack of exercise worsened feelings of anxiety and depression. Lower levels of sedentary time combined with higher levels of activity are also associated with better asthma control, reducing oxidative stress and improving quality of life [33].

This study has strengths and limitations. The strength is that these results are unique, given that similar studies on 6MWT and asthma assessment after biological therapy are lacking in the field. A limitation is the number of patients enrolled, which was small and restricted to Italian patients with severe asthma; therefore, these results are not generalisable to patients with less severe asthma or different ethnicities. In addition, we did not perform a 6MWT between baseline and 12 months; therefore, we cannot show how quick the improvement in 6MWD was, or if different patterns exist. In addition, this study was a pilot study and therefore further confirmation in larger populations is needed.

Conclusions

Our study analysed the possible relationship between severe eosinophilic asthma and 6MWT. The results show the potential of the 6MWT to complement severe asthma assessment, with 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 patients with severe asthma increased their 6MWD after initiation of therapy (both biological therapy with mepolizumab and conventional therapy), and this finding is in line with the few previous studies on this topic [24, 31, 34, 35]. 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, the 6MWT could be considered a relevant and practical tool in evaluating the objective response to severe asthma treatment. Given that the 6MWT is also a simple and inexpensive test that evaluates physical performance in a standardised way, it could be useful to extend use of the 6MWT in patients with severe asthma to longitudinally monitor in a more objective fashion the impact of biological therapies on asthma.

Provenance: Submitted article, peer reviewed.

Ethics statement: The study was approved by the local institutional review board (IRB number NP3364). All patients gave written informed consent for their data to be stored electronically.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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