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Treatment response according to small airways disease status: The effects of high-strength extrafine pMDI beclomethasone dipropionate/formoterol fumarate in fixed dose combination in moderate uncontrolled asthmatic patients

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ABSTRACT

Background: Inflammation in small airways is particularly clinically active in severe asthma but they still continue to be ignored as considered silent. Recently, the Atlantis study reports small airways involvement in 91% of the asthma population. Therefore in the era of phenotype driven therapy, the aim of this study was to verify if high-strength extrafine ICS/LABA in fixed dose increases clinical efficacy in moderate asthmatic patients with small airways dysfunction and it could be proposed as phenotype driven therapy.

Methods: In this prospective, non-interventional, real-life pilot study we enrolled 37 consecutive patients with moderate asthma who were uncontrolled despite GINA step 3 treatment. All subjects at enrollment were divided in two groups according to the presence of small airways dysfunction:1) small airways phenotype (SAP) group: smokers ($\geq$10 packs/die), ex-smokers ($>$ 20 packs/year) with air trapping (FVC $<$ 80% - VR $>$ 100% - FEF $_{25-75\%}$ $<$ 60%); 2) non-small airways phenotype (NSAP) group: non-smokers, without air trapping (FVC $\geq$80% - VR $\leq$ 100% - FEF $_{25-75\%}$ $\geq$60%). We later proceeded in both groups with a step up in therapy with high-strength extrafine pMDI beclomethasone dipropionate/formoterol fumarate (BDP/FF) (200/6 μg) in fixed dose to achieve a better control and followed patients for 6 months.

Results: Treatment with extrafine BDP/FF(200/6 μg) in SAP group showed a more significant improvement of FEF25-75%, FVC, RV, and a reduction of alveolar inflammatory markers such as FENO350 and alveolar exhaled pH compared with NSAP patients.

Conclusions: Our preliminary results support the use of high-strength extrafine pMDI BDP/FF (200/6 μg) as phenotype driven treatment directed to small airways dysfunction demonstrating an increase of clinical efficacy in moderate asthmatics with SAP.

1. Introduction

Uncontrolled asthma represents one of the most significant social and economic burden sworldwide consuming important resources in terms of direct and indirect costs [1]. Patients with moderate asthma present severe respiratory symptoms, frequent and severe exacerbations often requiring hospitalization, a functional decline and,overall, a very poor quality of life in terms of physical activity and social relationships that influence also their emotional sphere [2]. Accordingly to guidelines, if asthma is not adequately controlled on current therapies, treatment should be stepped up until control is achieved. The preferred treatment at step 5 before oral corticosteroids (OCS) and biologic therapy, is to combine a high dose of inhaled corticosteroids (ICS) pluslong acting bronchodilators (LABA) when control cannot be achieved with a medium dose of ICS combined with LABA, or with a high dose of ICS [3].

In the era of phenotype driven therapy, the extrafine formulations pMDI beclomethasone dipropionate/formoterolfumarate (BDP/FF)

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Abbreviations

ICS	inhaled corticosteroids
OCS	oral corticosteroids
LABA	long-acting beta-agonists
SABA	short-acting beta-agonists
SAP	Small airways phenotype
NSAP	Non small airways phenotype
SAD	small airways disease
NSAD	non-small airways disease
pMDI	“pressurized"Metered-Dose Inhaler
BDP/FF	beclometasone dipropionate/formoterol fumarate
FVC	forced vital capacity
FEV1	Forced expiratory volume in the 1st second
FEF	forced expiratory flow
TLC	total lung capacity
RV	residual volume
RFC	residual functional capacity
DLCO	diffusing capacity of lung for carbon monoxide;
FENO	fractional exhaled nitric oxide;
Ppb	parts per billion
Cig	cigarettes
COPD	chronic obstructive pulmonary disease
SPT	skin prick test
IgE	immunoglobulin E
TAI	Test of the Adherence to Inhalers
ACT	asthma control test
ACQ	Asthma Control Questionnaire;
AQLQ	Asthma Quality of Life Questionnaire;
EBC	exhaled breath condensate

might be considered as the best option when small airways involvement is evidenced [4,5]. However, the detection of small airways phenotype in order to guide the treatment decision is far from a common clinical practice and frequently this approach is responsible for treatment failure or an inappropriate and unnecessary step up to biologic therapy [6].

Most of the time we still consider the small airways as a “silent zone” [7–9], forgetting that small airways are clinically active, due to inflammation and remodeling, especially in patients with moderate asthma [10]. Recently, Usmani et al. reviewed several studies where all functional, biological and radiological techniques had been considered to explore the small airways, teaching us that more than 50% of asthmatics showed a small airways dysfunction [11]. An important change in the knowledge of small airways disease (SAD)role in asthma pathogenesis has been recently given by the Atlantis study [6]that reported a 91% prevalence of SAD features in asthma, defined as any abnormal measure on physiological tests including spirometry, body plethysmography, impulse oscillometry and multiple breath nitrogen washout. The cross-sectional phase of the study reported an increased small airways involvement in patients with a higher impact of the disease especially in GINA step 4 and 5, confirming that this anatomic zone is much more than silent.

Impairment of small airways can be of relevance in adult patients with moderate asthma, with a long duration of the disease and current or past smoking habit [12].Today the involvement of small airways can be diagnosed also with the use of non-invasive methods as the alveolar fractional exhaled nitric oxide (FENO) [13], the alveolar exhaled pH [14]and the collection of late-phase sputum [15] that give a further support to the recognized functional and radiologic tools usually used.

The aims of this preliminary study were to verify if high-strength extrafine ICS/LABA in fixed dose increases clinical efficacy in moderate asthmatic patients with small airways dysfunction in terms of clinical control and inflammatory profile, and to establish if this formulation could be proposed as phenotype driven therapy.

2. Methods

We conducted a prospective, observational, real-life,pilot study in 37 patients with moderate asthma consecutively enrolled through the outpatient clinic of severe asthma at the University Hospital of Foggia (Italy).

The study population was represented by adult patients with uncontrolled moderate asthma(ACQ score $>$ 1.5), classified asGINA step 4 [3], treated with medium dose ICS/LABA fixed combination as preferred controller and as-needed SABA for at least 4 weeks before entering the study, with an indication for a step up treatment with high dose of inhaled corticosteroid according to GINA guidelines, written informed consent was obtained from all subjects, and the institutional ethics committee of the University of Foggia approved the study(N° 17/CE/2014).

All asthmatics were assessed in a period of clinical stability and at least four weeks after an upper respiratory tract infection. Exclusion criteria were:chronic obstructive pulmonary disease (COPD), severe exacerbation or symptomatic infection in the previous 4 weeks, change in ICS dose in the previous 4 weeks.

All subjects at enrollment were divided in two groups (phenotypes) according to presence or not of small airways dysfunction:

- small airways phenotype (SAP) group:smokers ($\leq$10 cig/die) or former smokers ($<$ 20 pack/year), with air trapping (FVC $<$ 80% - VR $>$ 100% - FEF $_{25–75\%}$ $<$ 60%);
- non-small airways phenotype (NSAP)group: non-smokers, without air trapping (FVC $\geq$80% - VR $\leq$ 100% - FEF $_{25–75\%}$ $\geq$60%).

At the first visit (T0), complete baseline questionnaires, requesting information on medical history were administered to all subjects who were then given physical examination, atopy assessment, spirometry with bronchodilator reversibility test and pletismography. During the second visit (one day later), subjects underwent blood collection for inflammatory cells count, bronchial and alveolar fractional exhaled nitric oxide (FeNO) measurement. A subgroup of ten patients underwent also the exhaled breath condensate and induced sputum collection.

All subjects enrolled started high-strength extrafine pMDI beclomethasone dipropionate/formoterol fumarate (BDP/FF) (200/6 μg) in fixed dose at a daily dose of 800/24 μg as step up therapy.

At 3 and 6 months (T1 and T2) we recorded questionnaires, number of exacerbations and repeated functional tests and biological samples collections..

2.1. Atopic status

At baseline (T0), skin prick test (SPT) was performed for a panel of inhalant allergens as previously described for common aeroallergens (Lofarma, Italy) [16]. Total IGE were measured in blood [17,18].

2.2. Questionnaires

All patients completed different questionnaires at T0, T1 and T2:

- TAI-10: The therapeutic adherence of patients was evaluated by using adherence to inhalation therapies test (TAI - total score from 0 to 50), defining as poorly adhering to the therapy patients with scores below 45 [19].
- Asthma Quality of Life Questionnaire (AQLQ): evaluates the quality of life (score from 1 to 7), of which there is no univocal

interpretative cut-off but are taken into consideration the changes in the score obtained at the various follow-up visits, considering that numerically higher scores are associated with a better quality of life [20].

- Asthma control Questionnaire (ACQ) and Asthma control test (ACT) evaluate the levels of asthma control (score from 0 to 6 and from 0 to 25 respectively); they are indicative of poor control scores above 1.5 and scores below 20, respectively [21,22].

2.3. Lung function and pletismography

Pulmonary function tests were performed. Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were measured using a spirometer (Sensormedics, USA). The best value of three maneuvers was expressed as a percentage of the predicted normal value [23].After baseline evaluation, spirometry was repeated 15 min after the subjects had inhaled 400 μg of salbutamol as previously reported [23,24]. Reversibility of airways obstruction was expressed in terms of the percent changes from baseline of the forced expiratory volume in 1 s (FEV_1).All patients underwent pletismography (Sensormedics,USA)with the measure of residual volume (RV) and total lung capacity (TLC) [23].

2.4. Induced sputum collection and processing

According to the method described by Spanevello et al. [25]in moderate to severe asthmatics, sputum was induced through inhalation of hypertonic saline solution (4.5%) or isotonic saline solution (when the baseline FEV1 is < 60%)with an ultrasonic nebulizer (DeVilbiss65; DeVilbiss Corporation, Somerset, PA) and analyzed after selection of plug. Two plates were obtained in different steps of induction (the first after the first 5 min of nebulization considered early phase sputum; the second one, at the end of nebulization considered late phase sputum) [26].Only seven subjects were able to produce adequate sputum samples (defined as containing at least 500 non-squamous cells) [27].The sputum (the early and late phase) was used for cytological analysis.

2.5. Measurement of exhaled NO

Measurement of FeNO was performed according to recent guidelines [28].A rapid-response semi-portable NO analyzer (Medisoft FeNO + device) was used to quantify oxides of nitrogen (NOs). Two-point calibrations were performed daily using 5.2 parts per million calibration gas. Exhaled NO (FENO) was measured using a previously described restricted breath technique, which employed expiratory resistance and positive mouth pressure to close the velum and exclude nasal NO, and a constant expiratory flow of 50 mL/s (bronchial FENO) and a 350 mL/s (alveolar FENO). Repeated exhalations were performed until three plateaus agreed within 5%.

2.6. EBC collection and pH measurement in exhaled alveolar and bronchial condensate

1 mL of EBC was collected in one setting from 10 patients at the time of diagnosis, by using a condenser, which allowed for the non-invasive collection of non-gaseous components of the expiratory air (Turbo deck). We collected bronchial and separately alveolar EBC samples. Both condensates were collected on ice at −20 °C, aliquoted and stored at −70° for further analysis [29].

PH was measured on fresh samples, immediately after collection by means of a pH meter (FiveEasy Plus FP20, Mattler Toledo) with a −2 to 16.00 pH range and a resolution/accuracy of 0.01 ± 0.02 pH.

2.7. Statistical analysis

Descriptive statistics (i.e. means, standard deviations, percentages) were applied to summarize the continuous and categorical variables.

Table 1
Anthropometric, functional and clinical data in SAP and NSAP groups at T0.

	SAP (n = 19)	NSAP (n = 18)
Gender (female) (n, %)	12 (63%)	15 (83%)
Age (mean ± SD)	57.51 ± 14.18*	37.72 ± 9.22*
Atopy (n, %)	4 (21%)*	14 (78%)*
Age of onset (mean ± SD)	47.7 ± 5.9*	12.4 ± 9.6*
Currentsmokers (n, %)	4 (21%)*	0
Formersmokers (n, %)	15 (79%)*	0
Non-smokers (n, %)	0	18 (100%)*
TAI-10questionnaire (mean ± SD)	46 ± 2	47 ± 2
ACTquestionnaire (mean ± SD)	14.3 ± 1.9	17.9 ± 1.8
ACQquestionnaire (mean ± SD)	2.08 ± 0.72	2.13 ± 1.04
AQLQquestionnaire (mean ± SD)	4.65 ± 0.98	4.82 ± 1.16
Number of exacerbations/years (mean ± SD)	2.5 ± 0.3	2.7 ± 0.4
FVC (%)(mean ± SD)	72.1 ± 0.9*	99.4 ± 4.3*
FEV1 (%) pre-BD (mean ± SD)	66.4 ± 5.3*	100.9 ± 5.9*
FEV1 (%)post-BD (mean ± SD)	72.6 ± 6.1*	105.6 ± 6.1*
FEF 25–75 (%)(mean ± SD)	31.1 ± 3.7*	85.3 ± 2.4*
TLC (%)(mean ± SD)	98 ± 2.6	95 ± 2.1
RV (%)(mean ± SD)	138.6 ± 10.1*	95.5 ± 0.8*
RV/TLC	**41 ± 1.6***	**32 ± 2.5***
FRC (%)(mean ± SD)	**125.7 ± 9.6***	**96 ± 2.6***
FENO50 (ppb)(mean ± SD)	17.23 ± 2.5*	30.85 ± 7.3*
FENO350 (ppb) (mean ± SD)	15.4 ± 2.4	20.3 ± 3.4
Blood eosinophils (cell/microL) (mean ± SD)	130.3 ± 45.7*	270.8 ± 109.6*
Blood neutrophils (cell/microL) (mean ± SD)	**5.6 ± 2.7***	**3.8 ± 0.32***
Eosinophils in earlyphase sputum(mean ± SD)	2.9 ± 1.2*	4.4 ± 0.8*
Eosinophils in late phase sputum(mean ± SD)	3 ± 1.3*	4.7 ± 1.1*
Exhaled bronchial pH (mean ± SD)	7.3 ± 0.1	7.8 ± 0.1
Exhaled alveolar pH (mean ± SD)	6.7 ± 0.3	7.3 ± 0.2

***p < 0.005. Abbreviations:**SAP: small airways phenotype; NSAP: non-small airways phenotype; TAI: adherence to inhalation therapies test; ACT: asthma control test; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; FVC: forced vital capacity; FEV1: Forced expiratory volume in the 1st second; BD: bronchodilation; FEF: forced expiratory flow; TLC: total lung capacity; RV: Residual volume; **FRC: functional residual capacity**; FENO: fractional exhaled nitric oxide; ppb: parts per billion.

All variables analyzed were normally distributed. A Mann-Whitney *U* test was used to compare groups. Significance was defined as a *P* value of less than 0.05.

3. Results

Subjects included in the SAP group appeared older than the NSAP ones and affected by non atopic late-onset asthma.

Table 1 describe the main differences in baseline characteristics between SAP and NSAP groups.

After 6 months of treatment with extrafine pMDIBDP/FF 200/6 all asthmatics included showed a significant improvement of disease control (ACT and ACQ) and in terms of number of exacerbations/year (Table 2).

At T2of treatment the SAP group showed a significant higher FEF 25–75% (T0:31.1 ± 3.7 vs T2 49.7 ± 5.2, p = 0.05)(Table 2 - Fig. 1), a greater improvement of FVC (T0: 72.1 ± 0.9 vs T2: 92.5 ± 1.5, p = 0.02) (Table 2 - Fig. 2) and a significantly lower RV (T0: 138.6 ± 10.1 vs T2: 106.2 ± 4.0, p = 0.04)(Table 2 -Fig. 3) compared with NSAP patients.

Both groups demonstrated a significant improvement of alveolar FENO350 (SAP: T0 15.4 ± 2.4 vs T2 6.8 ± 1.1, p = 0.05; NSAP: T0 20.3 ± 3.4 vs T2 11.3 ± 2.2, p = 0.05) (Table 2 - Fig. 4 panel B), while in terms of bronchial FENO50they didn't showed significant changes(Table 2 - Fig. 4 panel A).

We collected 1 mL of EBC from 10 patients (5 of SAP group and 5 of

Table 2
Clinical, functional and biological parameters at T0 and after 6 months (T2) of therapy.

Variable (mean ± SD)	SAP			NSAP		
	T0	T2	P value	T0	T2	P value
TAI-10 questionnaire	46 ± 2	48 ± 1.9	Ns	47 ± 2	48 ± 0.5	ns
ACT questionnaire	14.3 ± 1.9	23.2 ± 1.8	**0.04**	17.9 ± 1.8	21.3 ± 1.4	**0.05**
ACQ questionnaire	2.08 ± 0.72	1.4 ± 0.2	**0.01**	2.13 ± 1.04	0.9 ± 0.5	**0.03**
AQLQ questionnaire	4.65 ± 0.98	6.12 ± 0.7	Ns	4.82 ± 1.16	5.9 ± 0.54	ns
Number of exacerbations/years	2.5 ± 0.3	0.7 ± 0.2	< **0.01**	2.7 ± 0.4	1.1 ± 0.4	**0.04**
FVC (%)	72.1 ± 0.9	92.5 ± 1.5	**0.02**	99.4 ± 4.3	102.9 ± 3.6	ns
FEV1 (%) pre-BD	66.4 ± 5.3	69.5 ± 3.4	Ns	100.9 ± 5.9	99.8 ± 2.8	ns
FEV1 (%)post-BD	72.6 ± 6.1	75.9 ± 5.2	Ns	105.6 ± 6.1	104.8 ± 3.1	ns
FEF 25–75% (%)	31.1 ± 3.7	49.7 ± 5.2	**0.05**	85.3 ± 2.4	92.4 ± 2.2	ns
TLC (%)	98 ± 2.6	96 ± 1.6	Ns	95 ± 2.1	93 ± 1.9	ns
RV (%)	138.6 ± 10.1	106.2 ± 4.0	**0.04**	95.5 ± 0.8	93.4 ± 0.9	ns
FENO50 (ppb)	17.23 ± 2.5	14.1 ± 2.3	Ns	30.85 ± 7.3	25.1 ± 5.0	ns
FENO350 (ppb)	15.4 ± 2.4	6.8 ± 1.1	**0.05**	20.3 ± 3.4	11.3 ± 2.2	**0.05**
Blood eosinophils (cell/microL)	130.3 ± 45.7	120.7 ± 14.2	Ns	270.8 ± 109.6	150.8 ± 91.2	< **0.01**
Eosinophils in earlysputum	2.9 ± 1.2	3.6 ± 0.7	Ns	4.4 ± 0.8	4.1 ± 0.7	ns
Eosinophils in late sputum	3.0 ± 1.3	3.3 ± 1.2	Ns	4.7 ± 1.1	3.6 ± 0.7	ns
Exhaledbronchial PH	7.3 ± 0.1	7.6 ± 02	Ns	7.8 ± 0.1	7.9 ± 0.1	ns
Exhaledalveolar PH	6.7 ± 0.3	7.5 ± 0.2	**0.02**	7.3 ± 0.2	7.4 ± 0.3	ns

Abbreviations: SAP: small airways phenotype; NSAP: **non-small** airways phenotype; n.s.:not significant; TAI: adherence to inhalation therapies test; ACT: asthma control test; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; FVC: forced vital capacity; FEV1: Forced expiratory volume in the 1st second; BD: bronchodilation; FEF: forced expiratory flow; RV: Residual volume; FENO: fractional exhaled nitric oxide; ppb: parts per billion.

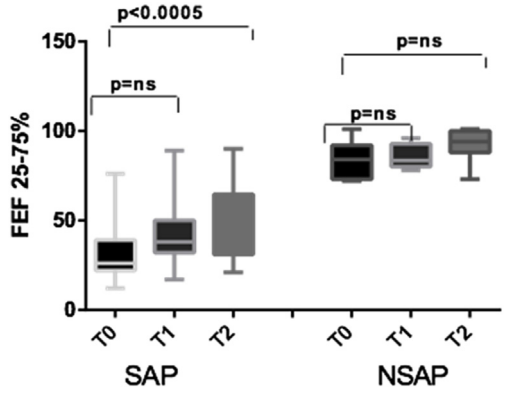

Fig. 1. Forced expiratory flow (FEF) 25–75% in small airways phenotype (SAP) and in non-small airways phenotype (NSAP) groups after 3 (T1) and 6 months (T2) of treatment with extrafine pMDI BDP/FF (200/6 μg).

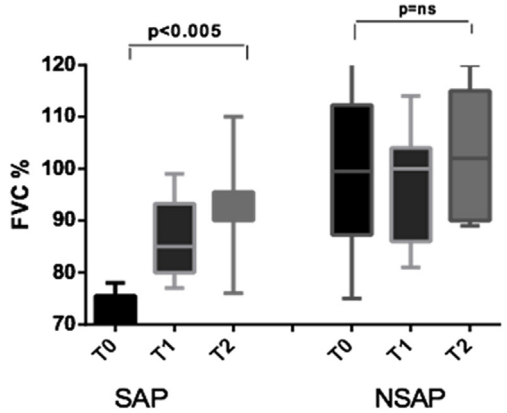

Fig. 2. Forced vital capacity (FVC) in small airways phenotype (SAP) and in non-small airways phenotype (NSAP) groups after 3 (T1) and 6 months (T2) of treatment with extrafine pMDI BDP/FF (200/6 μg).

NSAP group) and we measured pH in the bronchial and in the alveolar samples.Patients of SAP group showed a significant improvement in exhaled alveolar pH compared to NSAP ones (SAP: T0 6.7 ± 0.3 vs T2 7.5 ± 0.2, p = 0.05; NSAP: T0 7.3 ± 0.2 vs T2 7.4 ± 0.3,ns), while no differences appeared in terms of exhaled bronchial pH.

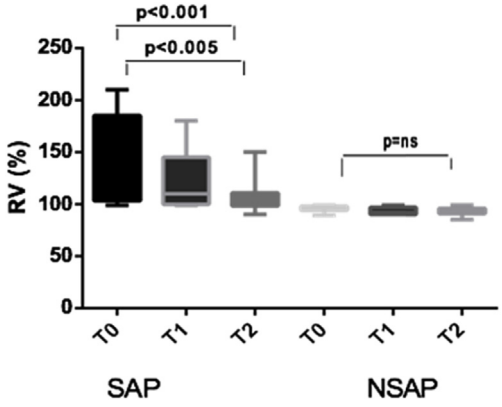

Fig. 3. Residual volume (RV) in small airways phenotype (SAP) and in non-small airways phenotype (NSAP) groups after 3 (T1) and 6 months (T2) of treatment with extrafine pMDI BDP/FF (200/6 μg).

Finally we analyzed blood and sputum eosinophils and after 6 months of treatment we recorded only a significant reduction of blood count in the NSAP group (T0: 270.8 ± 109.6 vs T2: 150.8 ± 91.2, p < 0.01).

4. Discussion

For the first time to our knowledge, our preliminary study focused on the efficacy of extrafine pMDI BDP/FF 200/6 in a specific group of moderate uncontrolled asthmatics with evidence of small airways dysfunction. This study confirms an important role of SAD in moderate uncontrolled asthma as evident by the impairment of functional and biological parameters that is expression of small airways involvement [30]. Patients belonging to the small airways phenotype showed to be more severe but also clinically different compared to those of non small airways phenotype as regard age, onset of the disease, smoking habit, lung function impairment [31].

Recently the Atlantis study [6]demonstrated that 91% of asthmatic subjects present an involvement of small airways defined as any abnormal physiological variable that couldn't be ignored. In this large

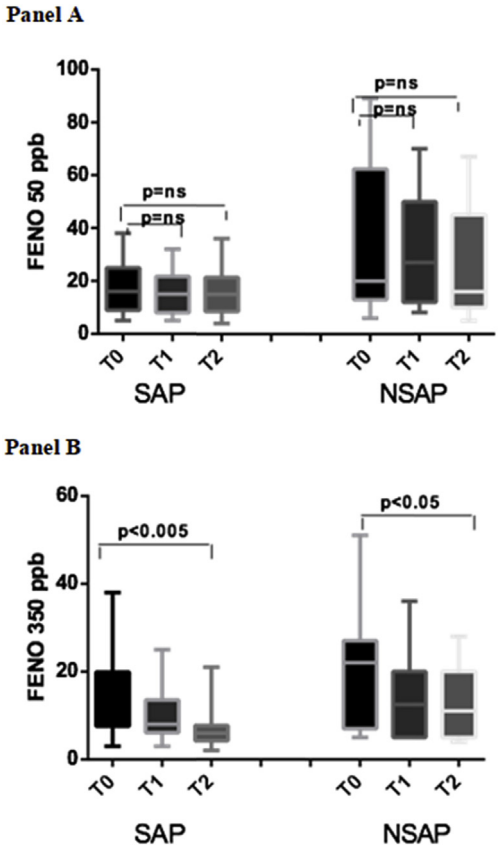

Fig. 4. Fractional exhaled nitric oxide (FENO) 50 (panel A) and FENO 350 (Panel B) in small airways phenotype (SAP) and in non-small airways phenotype (NSAP) after 3 (T1) and 6 months (T2) of treatment with extrafine pMDI BDP/FF (200/6 μg).

multicenter stud a real proportion of asthma patients suffering from SAD has been described and an important insight on which tests or combination of tests best allow to explore SAD has been given. Interestingly, the Atlantis study has contributed also to show the involvement of small airways in early and mild asthma, where the pathology starts, despite for a long time, we thought it was irrelevant. When something becomes evident, it cannot still be ignored. Therefore, after Atlantis we have a duty to explore SAD and treat it.

Considering the increased interest in the relationship between small airways impairment and the severity of asthma, in this study we aimed to deepen the knowledge on small airways disease pathology, in order to free small airways from the “silent zone” label. For the first time, we divided moderate uncontrolled asthmatic patients needing a step up therapy, in those with relevant involvement(small airways phenotype) and those without a small airways dysfunction hypothesizing that a phenotype driven treatment could have an increased clinically efficacy compared to those proposed by GINA guidelines Regarding patient's assignment to the SAP group, we used a classification routinely performer at our institution; we put a RV variable cut-off above 100% according to ERS/ATS reference [32]and a cut-point of 60% of predicted for FEF25-75% as lower values of FEF25-75% starting from an average 60% correlates with persistent symptoms, healthcare utilization in asthma, and biologic markers increment such as eNO, IgE, blood eosinophils and PC20 [33].

In our study, all subjects enrolled didn't differ in terms of control, quality of life and adherence to inhaled therapy but we found an important functional and inflammatory impairment of small airways only in the SAP patients.Differently from the Atlantis study we measured inflammatory markers in non-invasive samples as exhaled breath condensate and induced sputum. We confirmed an impairment of FEF 25–75%, FVC, and RV in our SAP patients, can be present already in mild asthma, but is prevalent in more compromised patients, becoming a complex and silent signature of those patients [15].

Moreover, this study shows that SAD phenotype is able to identify a different maybe highly prevalent population despite the similar disease clinical status, characterized by an older age and a later diagnosis, smoking history, less atopic status, more functional impairment.

As for lung function parameters [34–36], previous studies showed an increased alveolar FENO in severe and uncontrolled asthma, a correlation with functional parameter of peripheral airways dysfunction and finally a correlation with lung function decline as expression of its potentiality as prognostic marker [37].

For the first time, we described a more acid pH in alveolar breath condensate and more eosinophils in late phase sputum. In the past, Hoshino et al. [15], explored the possibility to have a biological photography of small airways proposing the cellular analysis of the late phase sputum. However, because of the need to produce enough sputum to be divided accordingly to anatomic site of production, this collection method has been ignored and not performed for years. The method allows, at the end of the procedure, to have a sample that mirrors the small airways. Just a small group of patients, equally divided in two phenotypes analyzed, were able to obtain bronchial and alveolar samples. As expected, eosinophils were higher in early sputum of all asthmatic subjects and particularly in NSAP where most ofthe patients were atopic. However, the percentage of eosinophils in late phase sputum was higher in SAP probably for a major involvement of small airways.

For the first time to our knowledge, wecollected breath condensate with a new device equipped with an alveolar valve that allows collection of bronchial and/or alveolar breath condensate. Also in this case, wecollected EBC from a small group of ten patients, equally divided in SAP and NSAP.

We measured a quick but validated inflammatory marker, the pH [38–40]that was proven more acid in alveolar EBC of SAP compared to NSAP [41]· The eosinophil inflammation showed locally and systemically in the SAP group, justifies a lower pH in those patients that is more evident in small airways samples, where the inflammation is higher.

It is therefore hypothesized that the use of extrafine formulations that ensure more uniform distribution throughout the entire bronchial tree, can improve small airway function, providing additional clinical benefits in terms of increased asthma control as compared to larger-particle formulations. In severe asthma, symptoms and exacerbations are more important as well as the involvement of small airways [42], therefore to treat patients with extrafine combination might be crucial.

In the effort to provide caregivers more flexibility to adapt treatments to specific patients’ condition, an extrafine formulation ICS/LABA, containing a higher dose of beclometasone dipropionate(200 μg/actuation) maintaining a standard doseofformoterolfumarate (6 μg/actuation) has been developed (Foster®, ChiesiFarmaceutici, Italy). This new fixed dose combination is characterized by an extrafine formulation of both active ingredients, which results in improved lung deposition and uniform treatment of asthma symptoms.

In this study, we analyzed the clinical, functional and biological effect of high-strength extrafine pMDI BDP/FF (200/6 μg) asa step up for moderate uncontrolled asthmatics.

As previously shown, extrafine pMDI BDP/FF (200/6 μg) determined a significant clinical improvement in asthmatic subjects [43,44].Patients belonging to both SAP and NSAP groups improved their ACT, ACQ, AQLQ, TAI scores and significantly decreased the number of exacerbations. This generally translates into greater asthma control and better quality of life when compared with larger particle ICS/LABA fixed combinations [45,46] in asthmatic patients not adequately controlled on high dose of ICS or medium dose of ICS in ICS/LABA combinations.In addition, the effect of extrafine pMDI BDP/FF (200/6 μg) on functional measures of small airways have been previously described and our results are perfectly in line, even compared to

medium strength ICS/LABA [42].

Therefore, we were able to confirm that patients belonging to SAP showed a significant improvement of clinical, functional and biological measures when treated with high-strength extrafine pMDI beclometasone dipropionate/formoterolfumarate (200/6 μg)compared to NSAP that anyway, also showed a good response to this combination. No studies, so far, exist on the effect of extrafine pMDI BDP/FF (200/6 μg) on biological small airways markers as FENO350, late phase sputum eosinophilsandpH in alveolar breath condensate. For the first time, we showed the effect of extrafine pMDI BDP/FF (200/6 μg) also on biological measures of small airways inflammation, reporting a reduction of both FENO350 and late phase sputum eosinophils and an increase of pH in alveolar breath condensate. The improvement of these biological tools was higher in SAP, where the involvement of small airways was important and the efficacy of extrafine pMDI BDP/FF was greater.

In this study, we have also overcome a limit of previous studies that investigated SAD,only testing one or a few physiologic SAD measures. For this reason, despite the small number of patients, we believe that the power of this study is to have focused on SAD, combining more physiological and biological measures that reflect small air ways involvement and to have analyzed the efficacy of extrafine pMDI BDP/FF on all of them.

In the SAP group we enrolled smokers and former smokers as agreed in the design of the study. We know that this choice could be represent an important bias related to small airways impairment and therefore we recognized it as a limit of the present study. The cigarette smoke represent in fact “per se” a factor that negatively affect small airways but this is a real life preliminary study whose interesting results deserve further confirming studies.

In conclusion, when moderate uncontrolled asthmatics with small airways phenotype are treated with high-strength extrafine pMDIBDP/FF (200/6 μg) that appear able to reach the main anatomic site of pathology, they obtain a very significant clinical improvement of asthma.

In the era of tailored treatments, it is mandatory to recognize specific phenotypes and to treat them with the best option available. This study further supports the importance to consider a simple or, if possible, a deepassessment to the small airways site, using traditional or novel measures giving a further contribution for a re-labeling of this anatomic zone. Note veryone can measure biomarkers in clinical practice to asses airways inflammation in small airways but the analysis of easy clinical characteristics that we selected to phenotype subjects in SAP could be proposed to identify those patients with small airways impairment and prescribe a more appropriate treatment.

Author contribution

GEC, GS and DL designed the study; GS, DL, PS, SS and CMIQ contributed to the clinical and laboratory work for the study; GEC, GS, DL, OR and MPFB drafted the article and revised it critically for important intellectual content; GEC, GS, and MPFB contributed to final approval of the version to be published. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Giovanna E. Carpagnano: Writing - original draft. **Giulia Scioscia:** Writing - original draft. **Donato Lacedonia:** Writing - original draft. **Silvia Romana Stornelli:** Data curation, Methodology, Writing - review & editing. **Carla Maria Irene Quarato:** Data curation, Methodology, Writing - review & editing. **Piera Soccio:** Methodology, Writing - review & editing. **Onofrio Resta:** Writing - original draft. **Maria Pia Foschino Barbaro:** Writing - original draft.

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