Methacholine Challenge Testing: Comparison of FEV_1 and Airway Resistance Parameters

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BACKGROUND: A 20% reduction in the FEV_1 is routinely used as an end point for methacholine challenge testing (MCT). Measurement of FEV_1 is effort dependent, and some patients are not able to perform acceptable and repeatable forced expiration maneuvers. The goal of the present study was to investigate the diagnostic value of airway resistance measurement by forced oscillation technique (FOT), body plethysmography, and interrupter technique compared with the traditionally accepted standard FEV_1 measurement in evaluating the responsiveness to methacholine during MCT. METHODS: We included in the study adult subjects referred for MCT because of asthma-like symptoms and with normal baseline spirometry. We modified routine MCT protocol by adding the assessment of airway resistance to the measurement of FEV_1 at each step of MCT. RESULTS: We observed, in the subjects with airway hyper-responsiveness versus those with normal airway responsiveness, a significantly greater percentage change in median (interquartile range) FOT resistance at 10 Hz (25.9% [13.7%–35.4%] vs 16% [15.7%–27.2%]), plethysmographic resistance (70.2% [39.5%–116.3%] vs 37.1% [23.9%–81.9%]), and mean ± SD conductance (−41.3 ± 15.4% vs −29.6 ± 15.9%); and a significantly greater change in mean ± SD FOT reactance at 10 Hz (–0.41 ± 0.48 cm H_2O/L/s vs –0.09 ± 0.32 cm H_2O/L/s) and at 15 Hz (–0.29 ± 0.2 cm H_2O/L/s vs –0.1 ± 0.19 cm H_2O/L/s). We also recorded significant differences in airway resistance parameters (FOT resistance at 10 Hz, FOT reactance at 15 Hz, plethysmographic airway resistance, and conductance indices as well as interrupter resistance) in FEV_1 non-responders at the onset of respiratory symptoms during MCT compared with baseline. CONCLUSIONS: Measurements of airway resistance could possibly be used as an alternative method to spirometry in airway challenge. Significant changes in airway mechanics during MCT are detectable by airway resistance measurement in FEV_1 non-responders with methacholine-induced asthma-like symptoms. (ClinicalTrials.gov registration NCT02343419.) *Key words: airway resistance; asthma; basic mechanisms; bronchial hyperresponsiveness; forced oscillation technique; interrupter technique; methacholine challenge; plethysmography.* [Respir Care 2021;66(3):449–459. © 2021 Daedalus Enterprises]

Introduction

Airway hyper-responsiveness (AHR) is one of the key features of asthma and is usually measured by direct airway challenges, for example, methacholine challenge testing (MCT).[1] Methacholine acts directly on smooth-muscle muscarinic receptors to induce bronchoconstriction.[2] In addition, direct action from histamine and indirect stimuli, such as exercise, eucapnic voluntary hyperpnea, and mannitol, are used in AHR evaluation.[2] Airway responsiveness to methacholine during MCT is recorded on the basis of the percentage decrease in FEV_1, which is measured repeatedly during challenge.[3,4]

FEV_1 measurement is an effort-dependent test that is influenced by many factors, including airway caliber and resistance as well as lung elastic recoil.[5] Furthermore, FEV_1 measurement requires patients' cooperation, and some patients are not able to perform acceptable and repeatable forced expiration maneuvers. The forced expiration maneuver is preceded by maximum inspiration, which may transiently decrease bronchoconstriction induced by methacholine inhalation.[3] Thus, there is an unmet need to assess AHR with an easy-to-perform, sensitive, and specific lung function test, which can be performed without deep inspiration.

Airway resistance, defined as the ratio of driving pressure and air flow, can be measured without forced respiratory

maneuvers and deep inhalations.[6] Forced oscillation technique (FOT), body plethysmography, and interrupter technique were previously used during MCT to measure airway resistance.[7-17] MCT guidelines[3] indicate airway resistance measurement as a possible alternative to spirometry in the evaluation of a response to a challenge agent; however, the lack of sufficient evidence is emphasized. The goal of the present study was to compare the diagnostic value of FOT, body plethysmography, and interrupter technique with the accepted standard FEV_1 measurement in evaluating responsiveness to methacholine during MCT.

QUICK LOOK

Current knowledge

Airway responsiveness to methacholine during methacholine challenge testing (MCT) is recorded on the basis of the percentage decrease in FEV_1. Measurement of FEV_1 is effort dependent, requires patients' cooperation, and some patients are not able to perform acceptable and repeatable forced expiration maneuvers.

What this paper contributes to our knowledge

In subjects with asthma-like symptoms and normal baseline spirometry, airway resistance parameters measured by the forced oscillation technique, body plethysmography, and interrupter technique were of acceptable diagnostic performance in identifying air-flow limitation, which resulted in a $\geq$ 20% decrease in FEV_1 during MCT. Significant changes in airway mechanics during MCT were detectable by airway resistance measurement in FEV_1 non-responders with methacholine-induced asthma-like symptoms.

Methods

Study Design

This cross-sectional study was performed in subjects referred to the lung function laboratory for MCT. In all the subjects, 2 sessions were arranged: (1) training session, and (2) MCT session. The maximum interval between the sessions was 7 d. During the training session, the subjects underwent a detailed medical history and physical examination. Validated pulmonary function testing techniques were used in the study.

Subjects

We included in the study consecutive adult subjects who presented to the pulmonary medicine out-patient clinic with asthma-like symptoms (cough, shortness of breath, wheezing, or chest tightness) and normal baseline spirometry. Exclusion criteria were as follows: (1) contraindications for airway challenge testing,[4] (2) respiratory infection in the 6 weeks before inclusion, and (3) administration of oral or inhaled corticosteroids in the 4 weeks before inclusion. The subjects were instructed to withhold antihistamines for 7 d before MCT and long-acting β-agonists for 48 h before MCT, and to avoid using short-acting bronchodilators or consuming coffee, tea, cola-type beverages, and chocolate within 24 h before MCT. We also asked the subjects to refrain from smoking on the day of the examination. All the subjects gave informed consent. The study protocol was approved by the Medical University of Warsaw bioethics board and was registered at ClinicalTrials.gov, NCT02343419.

Measurement of Fractional Concentration of Exhaled Nitric Oxide

The fractional concentration of exhaled nitric oxide (FeNO) was measured at the exhalation flow of 50 mL/s by using the FeNO+ system (Medisoft) according to American Thoracic Society/European Respiratory Society guidelines.[18] Measurement was performed on the day of MCT. Results are expressed as parts per billion.

MCT

Methacholine Dosage. MCT was performed according to the protocol based on the American Thoracic Society guidelines.[4] During the MCT, pulmonary function was assessed at (1) baseline, (2) after inhalation of normal saline solution (NSS), and (3) after inhalation of doubling the methacholine concentrations (from 0.03 to 16 mg/mL). Solutions were administered through 2-min continuous nebulization during tidal breathing. We used the ISPA provocation system (MES, Kracow, Poland) and the LC

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Plus nebulizer (PARI, Starnberg, Germany), powered by air at a pressure of 344 kPa.

Airway Response Assessment

We modified the American Thoracic Society protocol[4] by adding the assessment of airway resistance to the routine measurement of FEV_1 at each step of the MCT. Airway resistance was measured with FOT, body plethysmography, and the interrupter technique. Measurements were performed in a fixed sequence: (1) FOT, (2) body plethysmography, (3) interrupter technique, and (4) spirometry. Spirometry was performed last to avoid biasing the airway resistance measurement by possible transient bronchodilation. The sequence of airway resistance measurement techniques was set by considering the arrangement of the equipment in our laboratory to minimize the time of pulmonary function assessment. The interval between methacholine inhalation and the onset of FEV_1 measurement was within 3 to 6 min (5 [interquartile range {IQR} 4–5] min) and the interval between successive methacholine inhalations was within 7 to 12 min (9 [IQR 9–10] min). After each dose, the subjects were asked if they experienced the following asthma-like symptoms: cough, dyspnea, wheezing, and chest tightness.

After completion of MCT, the visual analog scale was used to assess subjects' perception of the difficulty of performing all 4 pulmonary function testing techniques used in the study. The scale was numbered from 0 (very easy) to 10 (extremely difficult). The MCT was discontinued in the following situations: (1) a decrease in FEV_1 after NSS inhalation of $\geq$20% compared with baseline, (2) a decrease in FEV_1 after the methacholine inhalation of $\geq$20% compared with the NSS step, and (3) inhalation of the highest concentration of methacholine (16 mg/mL). In the case of situation (1) or (2), 200 μg of salbutamol was administered by inhalation, and pulmonary function tests were performed after 15 min to confirm the resolution of bronchial obstruction.

Pulmonary Function Testing Techniques

Spirometry. FEV_1 was measured by using the LungTest 1000 spirometer (MES) according to American Thoracic Society/European Respiratory Society recommendations.[19] We used 30-mm-diameter disposable paper mouthpieces (Naturfarm, Poznan, Poland) and reusable, sterilizable DV 40 pneumotachographs (MES).

FOT

FOT resistance (R_{FOT}) and FOT reactance (X_{FOT}) were measured with the use of Micro 5000 Rosc equipment (Medisoft, Sorinnes, Belgium) according to European Respiratory Society Task Force guidelines.[20] R_{FOT} and X_{FOT} were measured with the following oscillation frequencies: 5, 10, 15, 20, 25, and 30 Hz (R_{FOT_5}, R_{FOT_10}, R_{FOT_15}, R_{FOT_20}, R_{FOT_25}, R_{FOT_30}, respectively; and X_{FOT_5}, X_{FOT_10}, X_{FOT_15}, X_{FOT_20}, X_{FOT_25}, X_{FOT_30}, respectively). At each frequency, after stabilization of the breathing frequency and tidal volume, we recorded 10 s of measurement. On the basis of data from the existing literature [7,9,21,22] and results of the interim analysis of our results, we recognized R_{FOT_5}, R_{FOT_10}, R_{FOT_15}, and the difference between R_{FOT_5} and R_{FOT_20} (R_{FOT_5-20}) as the best indicators of changes in airway function and only those FOT indices were included in the final analysis.

Body Plethysmography

We measured plethysmographic airway resistance (R_{aw}), airway conductance (G_{aw}), specific airway resistance (sR_{aw}), and specific airway conductance (sG_{aw}) by using BodyBox 5500 cabin plethysmograph (Medisoft). Measurements were obtained according to the principles described by Goldman et al.[23] At each step of MCT, the mean value of $\geq$5 repeatable measurements was recorded.

Interrupter Technique

The interrupter resistance (R_{INT}) was measured by using dedicated module of BodyBox 5500 plethysmograph (Medisoft). Measurement was performed according to the recommendation provided by the European Respiratory Society Task Force.[24] At each step of MCT, the mean value of $\geq$5 repeatable R_{INT} measurements was recorded.

Statistical Methods

Continuous variables with normal distribution are presented as mean $\pm$ SD, those non-normally distributed are presented as median (IQR). Changes in FEV_1 and all airway resistance parameters except X_{FOT} are expressed as the percentage of the NSS step value. Changes in X_{FOT} are expressed as absolute numbers. As previously reported, expressions of changes in X_{FOT} as a percentage may result in unrealistic numbers, because X_{FOT} values range from negative to positive and cross zero.[9]

We used the Student *t* test and the Mann-Whitney U test to assess the differences between the 2 groups in normally and non-normally distributed variables, respectively. Differences in categorical variables between the 2 groups were assessed by using the chi-square test. Differences in pulmonary function indices at baseline and at the onset of asthma-like symptoms were assessed by using the dependent Student *t* test and the Wilcoxon test for normally and non-normally distributed variables, respectively. The Benjamini-Hochberg adjustment procedure with the false

discovery rate set at 10% was used to correct for multiple testing.

We used receiver operating characteristic curves for the assessment of the diagnostic yield of the airway resistance parameters in the diagnosis of air-flow limitation that causes a 20% FEV_1 decrease. The optimum cutoff values of the change in the airway resistance indices were determined on the basis of receiver operating characteristic curve analysis at the highest Youden index.[25] We also calculated the sensitivity and specificity as well as diagnostic odds ratio[26] of the airway resistance parameters in detecting a 20% FEV_1 decrease when using previously proposed cutoff values.[4,10,15,23,27-29] Calculations were performed by using the data analysis software system Statistica version 13 (TIBCO Software, Palo Alto, California). $P < .05$ was considered statistically significant.

Results

Subjects

Of the 49 subjects enrolled in the study, 7 (14.3%) were not included in the final analysis for the following reasons: the inability to perform repeatable FEV_1 measurements ($n = 3$), withdrawal of consent after the training session ($n = 2$), severe coughing paroxysms during spirometric maneuvers ($n = 1$), and a ≥ 20% fall in FEV_1 compared with baseline after inhalation of NSS ($n = 1$). Baseline characteristics of the 42 study completers and 7 dropouts are presented in Table 1. The dropouts did not differ significantly from the completers with respect to demographic characteristics and comorbidities. Spirometry was within the normal range in all of the study completers.

Table 1. Characteristics of Study Group

Characteristic	Completers ($n = 42$)	Dropouts ($n = 7$)
Demographic characteristics		
Sex distribution, no. women (%)	21 (50)	6 (85.7)
Age, median (IQR) y	36 (30–56)	39 (35–64)
BMI, mean ± SD kg/m^2	25.8 ± 4.1	26.9 ± 5.8
Comorbidities, *n* (%)		
Arteria hypertension	9 (21.4)	1 (14.3)
Chronic sinusitis	7 (16.7)	1 (14.3)
GERD	6 (14.3)	0
Allergic rhinitis	4 (9.5)	0
Smoking status, *n* (%)		
Smokers	5 (11.9)	1 (14.3)
Ex-smokers	5 (11.9)	2 (28.6)
Non-smokers	32 (76.2)	4 (57)
Baseline spirometry: FEV_1, mean ± SD L	3.38 ± 0.88	NA
FEV_1 % predicted, mean ± SD %	93.9 ± 13.9	NA
FVC, mean ± SD L	4.4 ± 1.1	NA
FVC % predicted, mean ± SD %	98.3 ± 13.7	NA

IQR = interquartile range
BMI = body mass index
GERD = gastroesophageal reflux disease
NA = not applicable

MCT

Airway Responsiveness to Methacholine. In 25 subjects (59.5%), we recorded a ≥ 20% fall in FEV_1 during MCT (FEV_1 responders [R group]), and, in 17 subjects (40.5%), the fall in FEV_1 was < 20% (FEV_1 non-responders [NR group]). We present the results of the MCT and the process of inclusion in the study groups in Figure 1. There were no significant differences in baseline spirometry and airway resistance parameters between the R and NR groups (data available in Table 2, described herein). The median (IQR) pre-test FeNO was significantly higher in the subjects from the R group versus the NR group (34 [19-49.5] ppb vs 18 [10-30] ppb; $P = .009$).

FEV_1 and Airway Resistance Parameters

The comparison of changes in the FEV_1 and airway resistance parameters between the NSS step and the final step of MCT in the R and NR groups are presented in Table 3. We observed a significantly greater median (IQR) percentage change in R_{FOT_10} (25.9% [13.7%–35.4%] vs 16% [15.7%–27.2%]; $P = .042$) and R_{aw} (70.2% [39.5%–116.3%] vs 37.1% [23.9%–81.9%]; $P = .032$), and the mean ± SD G_{aw} (–41.3% ± 15.4% vs –29.6% ± 15.9%; $P = .02$) as well as a significantly greater change in the mean ± SD X_{FOT_10} (–0.41 ± .48 cm H_2O/L/s vs –0.09 ± 0.32 cm H_2O/L/s; $P = .02$) and mean ± SD X_{FOT_15} (–0.29 ± 0.2 cm H_2O/L/s vs –0.1 ± 0.19 cm H_2O/L/s; $P = .003$) in the subjects with AHR diagnosed on the basis of a ≥ 20% change in FEV_1 during MCT versus those with normal airway responsiveness.

In Figure 2, we present the individual profile plots for the subjects in the R group and the NR group, and report values of FEV_1 and selected airway resistance parameters at the NSS step and the final step of MCT. We noted that, in both R and NR groups, all resistance parameters, except R_{FOT_5}, were significantly higher and that G_{aw} as well as sG_{aw} were significantly lower at the end of MCT compared with the NSS step. Furthermore, in the R group, X_{FOT_10} and X_{FOT_15} were significantly lower at the end of MCT compared with the NSS step, whereas, in the NR group, none of the reactance parameters differed significantly between the NSS step and the final step of MCT. The sensitivity and specificity of the selected previously proposed cutoff values of changes in airway resistance parameters for the detection of air-flow limitation that causes a ≥ 20% decrease in FEV_1 are shown in Table 4. The optimum cutoff values of a change in airway resistance indices for the detection of air-flow limitation that results in a ≥ 20% decrease in FEV_1, determined on the

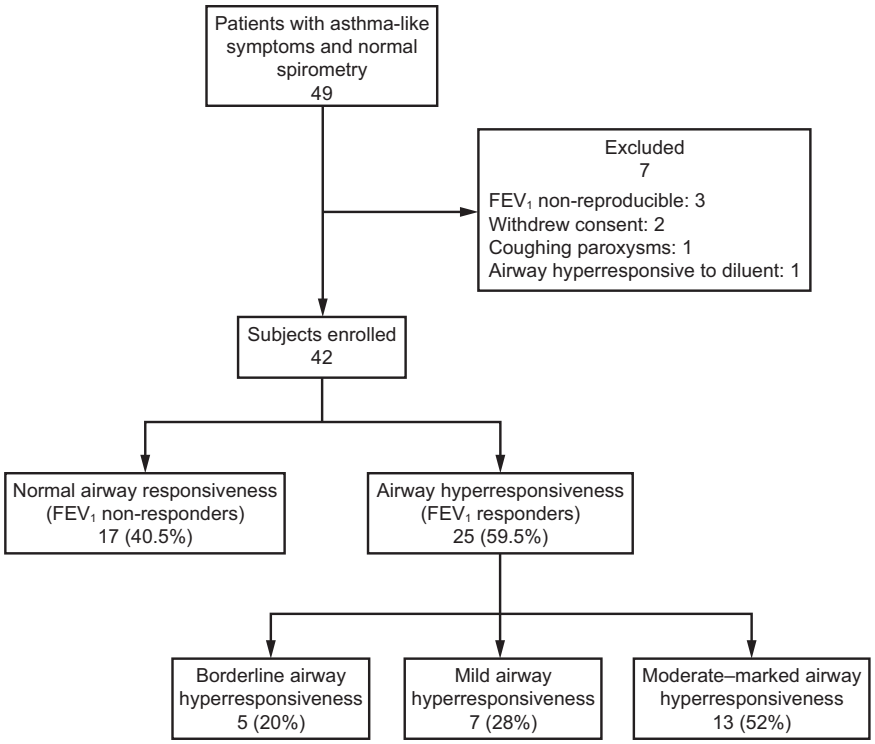

Fig. 1. Flow chart

basis of receiver operating characteristic curve analysis, are presented in Table 5 (only parameters for which the area under the curve was > 0.8 are included).

Asthma-Like Symptoms during MCT

The occurrence of at least one of the asthma-like symptoms was observed in 34 subjects (81%) during MCT. Cough was recorded in 31 subjects (73.8%). We also observed wheezing ($n = 9$ [21.4%]), dyspnea ($n = 5$ [11.9%]), and chest tightness ($n = 2$ [4.8%]). Asthma-like symptoms occurred with similar frequency in both R and NR groups: at least 1 symptom occurred in 21 subjects (84%) from R group and 13 subjects (76.5%) form NR group ($P = .50$). In 5 subjects, asthma-like symptoms occurred after forced expiration during baseline spirometry (3 subjects from the R group and 2 subjects from the NR group). The comparison of pulmonary function indices at the onset of symptoms with baseline values in the remaining 29 subjects who were symptomatic in the R and NR groups are presented in Table 2. We observed significant differences in the airway resistance parameters at the onset of asthma-like symptoms compared with the baseline, not only in symptomatic FEV_1 responders, but we also recorded significant differences in R_{FOT_10}, X_{FOT_15}, R_{aw}, sR_{aw}, sG_{aw}, and R_{INT} in FEV_1 non-responders who were symptomatic.

Subjects' Perception of the Difficulty of Pulmonary Function Tests

The visual analog scale scores for subject ratings of procedural difficulty differed significantly among different pulmonary function tests ($P < .001$). We recorded the following median (IQR) visual analog scale scores for spirometry, FOT, plethysmography, and interrupter technique: 4 (2–6), 0 (0–0), 1 (0–2), and 0 (0–0), respectively. The subjects perceived spirometry as significantly more difficult compared with FOT (post hoc, $P < .001$), plethysmography (post hoc, $P < .001$), and interrupter technique (post hoc, $P < .001$). Furthermore, the visual analog scale score for plethysmography was significantly greater compared with both the FOT (post hoc, $P < .001$) and interrupter technique (post hoc, $P < .001$).

Discussion

We confirmed the usefulness of 3 airway resistance measurement techniques in the assessment of airway responsiveness to methacholine. We observed a significantly greater increase in R_{FOT_10} and R_{aw}, and a decrease in X_{FOT_10}, X_{FOT_15}, and G_{aw} at the end of the MCT in the subjects with AHR, defined on the basis of a $\geq$ 20% decrease in FEV_1 during the MCT compared with those with normal airway responsiveness. We found that the airway resistance parameters measured by FOT and the interrupter technique as well as the airway resistance and conductance parameters measured

Table 2. Pulmonary Function Indices at Baseline and at the Onset of Respiratory Symptoms During MCT

Parameter	Baseline	Symptoms Onset	*P*
FEV_1 responders with asthma-like symptoms induced by MCT ($n = 18$)			
FEV_1, mean (IQR), L	3.26 ± 0.86	2.88 ± 0.82	<.001*
R_{FOT_5}, median (IQR) cm H_2O/L/s	9.29 (8.22–12.73)	13 (11.28–14.3)	.002*
R_{FOT_10}, mean ± SD cm H_2O/L/s	4.48 ± 0.54	5.25 ± 0.79	<.001*
R_{FOT_15}, median (IQR) cm H_2O/L/s	4.07 (3.88–4.35)	4.64 (4.18–5.48)	.002*
$R_{FOT\_5–20}$, median (IQR) cm H_2O/L/s	5.24 (4.53–8.97)	8.42 (7.00–1.44)	.003*
X_{FOT_5}, mean ± SD cm H_2O/L/s	1.59 ± 0.98	1.64 ± 1.22	.62
X_{FOT_10}, mean ± SD cm H_2O/L/s	–0.85 ± 0.44	–1.12 ± 0.59	.01
X_{FOT_15}, median (IQR) cm H_2O/L/s	–0.39 (–0.61 to –0.32)	–0.68 (–0.8 to –0.46)	.01*
R_{aw}, mean ± SD cm H_2O/L/s	1.77 ± 0.55	2.55 ± 0.85	<.001*
G_{aw}, median (IQR) L/s/cm H_2O	0.59 (0.52–0.7)	0.39 (0.34–0.48)	.003*
sR_{aw}, mean ± SD cm H_2O·s	7.08 ± 2.16	1.49 ± 3.66	<.001*
sG_{aw}, mean ± SD 1/s/cm H_2O	0.15 ± 0.05	0.11 ± 0.04	.002*
R_{INT}, mean ± SD cm H_2O/L/s	3.51 ± 0.88	4.1 ± 1.19	.008*
FEV_1 non-responders with asthma-like symptoms induced by the MCT ($n = 11$)			
FEV_1, mean ± SD L	3.63 ± 1.04	3.53 ± 0.96	.12
R_{FOT_5}, mean ± SD cm H_2O/L/s	11.77 ± 2.16	12.43 ± 3.62	.55
R_{FOT_10}, mean ± SD cm H_2O/L/s	4.59 ± 0.71	5.06 ± 0.7	.069*
R_{FOT_15}, mean ±SD cm H_2O/L/s	4.15 ± 0.57	4.56 ± 0.49	.09
$R_{FOT\_5–20}$, mean ± SD cm H_2O/L/s	7.99 ± 2.08	8.23 ± 3.80	.84
X_{FOT_5}, mean ± SD cm H_2O/L/s	1.68 ± 1.01	1.64 ± 1.36	> .99
X_{FOT_10}, mean (±SD), cm H_2O/L/s	–0.84 ± 0.52	–0.92 ± 0.72	.66
X_{FOT_15}, median (IQR) cm H_2O/L/s	–0.55 (–0.74 to –0.23)	–0.59 (–0.76 to –0.36)	.02*
R_{aw}, mean ± SD cm H_2O/L/s	1.77 ± 0.56	2.15 ± 0.78	.02*
G_{aw}, mean ± SD L/s/cm H_2O	0.62 ± 0.18	0.53 ± 0.2	.08
sR_{aw}, mean ± SD cm H_2O×s	5.88 ±3.05	8.21 ± 4.41	.006*
sG_{aw}, mean ± SD 1/s/cm H_2O	0.21 ± 0.11	0.16 ± 0.09	.03*
R_{INT}, mean ± SD cm H_2O/L/s	3.24 ± 0.68	4.07 ± 1.07	.01*

* *P* remains significant after correction when using the false discovery rate < 10% for comparison of the measurement at baseline and at the onset of respiratory symptoms. MCT = methacholine challenge testing; IQR = interquartile range; R_{FOT_5} = forced oscillation resistance at 5 Hz; R_{FOT_10} = forced oscillation resistance at 10 Hz; R_{FOT_15} = forced oscillation resistance at 15 Hz; $R_{FOT\_5–20}$ = the difference between R_{FOT_5} and R_{FOT_20}; X_{FOT_5} = forced oscillation reactance at 5 Hz; X_{FOT_10} = forced oscillation reactance at 10 Hz; X_{FOT_15} = forced oscillation reactance at 15 Hz; R_{aw} = airway resistance (measured by using body plethysmography); G_{aw} = airway conductance (measured by using body plethysmography); sR_{aw} = specific airway resistance; sG_{aw} = specific airway conductance (measured by using body plethysmography); R_{INT} = interrupter resistance.

by plethysmography differed significantly at the end of MCT compared with NSS both in the FEV_1 responders and non-responders. However, X_{FOT_10} and X_{FOT_15} were significantly lower at the end of the MCT compared with NSS in the FEV_1 responders but did not differ significantly in the FEV_1 non-responders. We also showed that the occurrence of respiratory symptoms in the FEV_1 non-responders during the MCT was related to significant changes in airway resistance parameters.

Our work was important for several reasons. First, the demonstration of the usefulness of the airway resistance measurement during MCT indicates the possibility of implementing it as an alternative to spirometry, especially in patients not able to perform numerous, repeated forced expiration maneuvers. All of the applied airway resistance measurement methods, even including plethysmography, were rated by the study subjects as easier to perform compared with spirometry. Second, the occurrence of symptoms indicative of air-flow limitation and significant changes in airway resistance parameters in the FEV_1 non-responders indicated that AHR assessment with the use of only FEV_1 may be insufficient. The idea of applying airway resistance measurements to diagnose AHR is not new. However, the evidence for the diagnostic value of those measurements has largely been lacking. To the best of our knowledge, this was the first study in which 3 airway resistance measurement methods and spirometry were used simultaneously during MCT in adults. Our approach allowed a direct comparison among all 4 techniques.

Achievement of an accurate, reproducible FEV_1 measurement may be problematic for some patients and the inability to perform repeatable FEV_1 measurements was the most common reason for dropouts in our cohort (6.1% of enrolled subjects). Furthermore, one subject was excluded from the study due to paroxysmal cough during the first second of forced expiration. In this subject, no cough paroxysms were observed during airway resistance measurement by FOT, plethysmography, and the interrupter technique. A comparable percentage

Table 3. Changes in FEV_1 and Airway Resistance Parameters between the Normal Saline Solution Step and the Final Step of Methacholine Challenge Testing

Change	FEV_1 Responders ($n = 25$)	FEV_1 Non-Responders ($n = 17$)	P
%Δ FEV_1, median (IQR)	–25.8 (–27.9 to –22.8)	–11.4 (–15.3 to –9.)	
%Δ R_{FOT_5}, mean ± SD	13.9 ± 36.1	2.9 ± 30.4	.31
%Δ R_{FOT_10}, median (IQR)	25.9 (13.7–35.4)	16 (15.7–27.2)	.042*
%Δ R_{FOT_15}, median (IQR)	29.8 (18.8–41.6)	22.6 (15.7–27.2)	.14
%Δ $R_{FOT\_5–20}$, median (IQR)	7.8 (−20.7 to 28.3)	−10.4 (−39.0 to 14.3)	.24
ΔX_{FOT_5}, median (IQR) cm H_2O/L/s	–0.06 (–0.52 to 0.5)	0.42 (–0.64 to 1.32)	.58
ΔX_{FOT_10}, mean ± SD cm H_2O/L/s	–0.41 ± 0.48	–0.09 ± 0.32	.02*
ΔX_{FOT_15}, mean ± SD cm H_2O/L/s	–0.29 ± 0.2	–0.1 ± 0.19	.003*
%Δ R_{aw}, median (IQR)	70.2 (39.5–116.3)	37.1 (23.9–81.9)	.032*
%Δ G_{aw}, mean ± SD	–41.3 ± 15.4	–29.6 ± 15.9	.02*
%Δ sR_{aw}, median (IQR)	95.3 (53.9–15.8)	58.8 (31.7–96.1)	.17
%Δ sG_{aw}, mean ± SD	–46.7 ± 18	–4.2 ± 16.7	.24
%Δ R_{INT}, median (IQR)	49.8 (4.8–61.4)	38.9 (26.1–85.4)	.64

* P remains significant after correction when using the false discovery rate of <10% for comparison of responders vs non-responders. %Δ = percentage change; IQR = interquartile range; R_{FOT_5} = forced oscillation resistance at 5 Hz; R_{FOT_10} = forced oscillation resistance at 10 Hz; R_{FOT_15} = forced oscillation resistance at 15 Hz; $R_{FOT\_5–20}$ = the difference between R_{FOT_5} and R_{FOT_20}; Δ = absolute change; X_{FOT_5} = forced oscillation reactance at 5 Hz; X_{FOT_10} = forced oscillation reactance at 10 Hz; X_{FOT_15} = forced oscillation reactance at 15 Hz; R_{aw} = airway resistance (measured by using body plethysmography); G_{aw} = airway conductance (measured by using body plethysmography); sR_{aw} = specific airway resistance; sG_{aw} = specific airway conductance (measured by using body plethysmography); R_{INT} = interrupter resistance.

of non-repeatable FEV_1 measurements were observed by Enright et al,[30] who noted that 5% of 18,000 consecutive adults subjects referred to an out-patient pulmonary function laboratory for spirometry were unable to match their highest FEV_1 within 150 mL. In such patients, the airway resistance measurement could be the only feasible method of assessing the response to airway challenge.

Furthermore, we believe that the airway resistance measurement could be used as a complementary method to detect excessive changes in airway function during MCT in FEV_1 non-responders, especially in patients with typical asthma-like symptoms induced by methacholine. FEV_1 mainly reflects the function of proximal airways.[31] In asthma, the inflammatory process also extends to the peripheral airways.[32] Changes in small-airways function result in changes in R_{FOT} measured at low frequencies (5–15 Hz), and increased $R_{FOT\_5–20}$ was reported to reflect the abnormal function of small airways.[33,34] The function of peripheral airways is also reflected in sG_{aw} and sR_{aw}, measured by body plethysmography.[35] Moreover, FEV_1 could underestimate the responsiveness to methacholine due to the bronchodilatory effect of deep breathing.[3] Prominent changes in the airway resistance parameters in patients without a 20% decrease in FEV_1 during MCT may help to support an asthma diagnosis and avoid the performance of a burdensome workup for other diseases that can mimic asthma. Moreover, establishing the diagnostic value of airway resistance as the marker of AHR could possibly lighten the burden imposed on patients by repeated pulmonary function assessment during MCT.

The usefulness of FOT, body plethysmography, and interrupter technique in the assessment of the airway response to methacholine was previously assessed in a few studies. Yoon et al[7] and Short et al[8] found that the mean increase of R_{FOT_5} at the end of MCT was 31.5% in children with asthma and 43.5% in adults with asthma, respectively. R_{FOT_5} was also reported to correlate better with respiratory symptoms during MCT compared with FEV_1.[21] In our study, the mean change in R_{FOT_5} at the end of MCT in the subjects with AHR was only 13.9%. We also noticed a median increase in $R_{FOT\_5–20}$ of only 7.8% during MCT in the subjects with AHR, which is much less pronounced than that recorded by Short et al[8] in the subjects with asthma (mean percentage increase during MCT of 272.2%). Discrepancies may result from the use of a different FOT apparatus by the investigators in the above-mentioned studies and in our study. We observed prominent sinusoidal fluctuations of R_{FOT_5} with the breathing cycle; such fluctuations were not visible when measuring R_{FOT} at higher frequencies. Similar difficulties were previosly described by King et al., who attributed them to the closeness of breathing and oscillation frequencies.[36]

Furthermore, we noted that a set of airway resistance parameters measured by FOT, body plethysmography, and the interrupter technique differed significantly between the NSS and the final step of MCT in the subjects with AHR and normal airway responsiveness, whereas reactance measured by FOT at 10 and 15 Hz differed significantly only in the subjects with AHR. In line with our findings, X_{FOT} was previously shown to be better correlated with FEV_1 compared with R_{FOT}, and the dose-response slope of

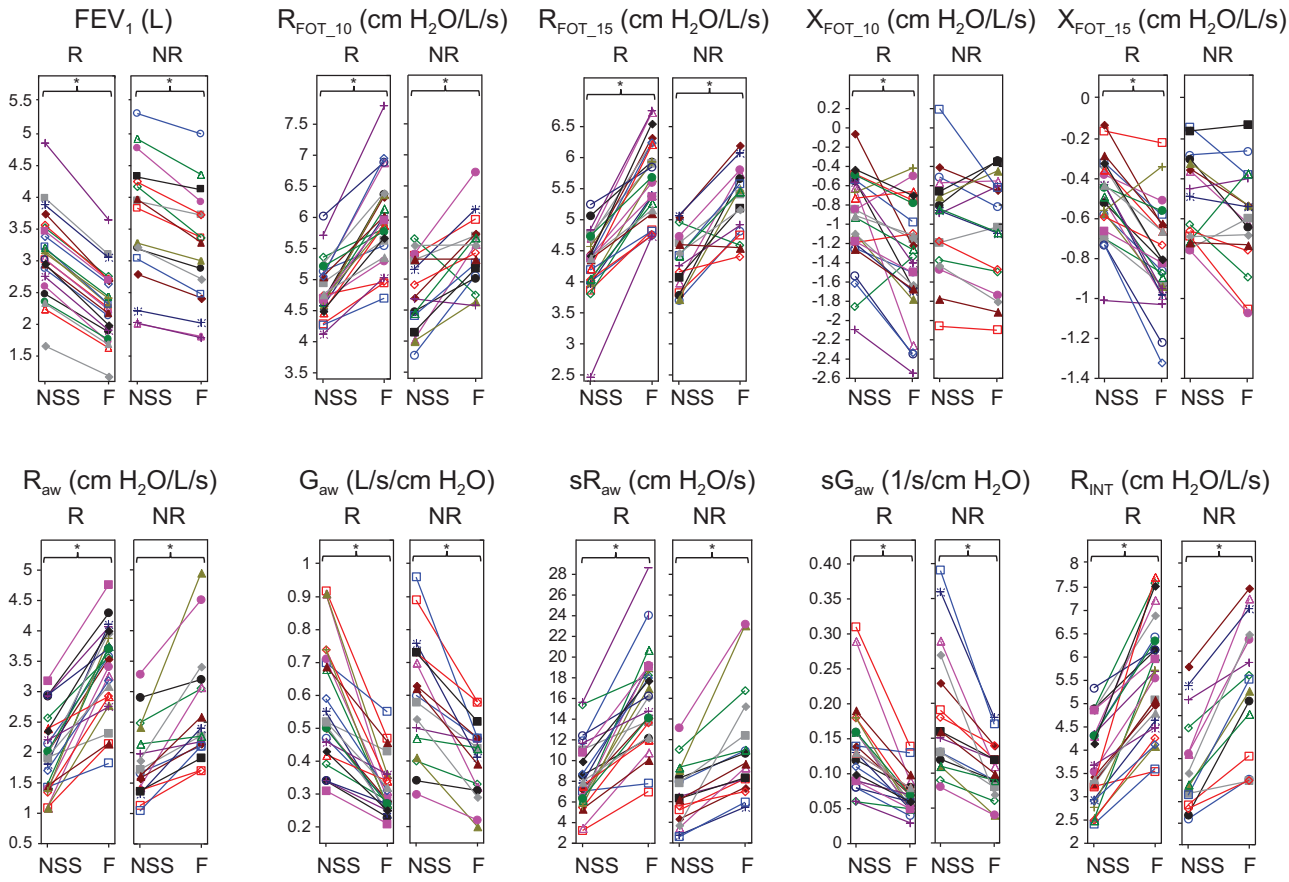

Fig. 2. Comparison of selected pulmonary function parameters at the normal saline solution (NSS) step and final (F) step of methacholine challenge testing in the FEV_1 responders (R group) and FEV_1 non-responders (NR group). * $P < .05$. Each line represents an individual subject.

Table 4. Diagnostic Performance of Selected Cutoff Values of Changes in Airway Resistance Parameters for the Detection of Air-Flow Limitation that Resulted in a Decrease in FEV_1 of at Least 20%

R_{aw} Parameter	Cutoff Value	Sensitivity, %	Specificity, %	DOR (95% CI)
R_{FOT_5}	≥25% increase	32	77.2	1.6 (0.7–3.8)
R_{FOT_10}	≥30% increase	40	95.9	15.7 (5.8–42)
R_{aw}	≥50% increase	60	8.8	6.3 (2.7–14.8)
Specific R_{aw}	≥40% increase	80	64.7	7.3 (2.7–2.1)
Specific R_{aw}	≥45% increase	76	68.5	6.8 (2.7–17.8)
sG_{aw}	≥35% decrease	76	75.3	9.7 (3.7–25.2)
sG_{aw}	≥40% decrease	68	81.2	9.2 (3.8–22.3)
sG_{aw}	≥45% decrease	60	86.6	9.7 (4.1–23.2)
R_{INT}	≥35% increase	76	78.1	11.3 (4.3–29.5)

R_{aw} = airway resistance; DOR = diagnostic odds ratio; R_{FOT_5} = forced oscillation resistance at 5 Hz; R_{FOT_10} = forced oscillation resistance at 10 Hz; sG_{aw} = specific airway conductance; R_{INT} = interrupter resistance.

X_{FOT} in MCT was shown to better differentiate the subjects with asthma from healthy subjects compared with the dose-response slope of R_{FOT}.[7,37]

Also, plethysmographic sG_{aw} was previously evaluated as a predictor of a ≥ 20% decrease in FEV_1 during MCT. Higher sensitivity (89%) and lower specificity (55%) were reported by Khalid et al[11] compared with our data. Kraemer et al[10] compared plethysmographic sG_{aw} and FEV_1 diagnostic accuracy in differentiating subjects with asthma from subjects without asthma. They reported that a ≥ 40% decrease in sG_{aw} allows the diagnosis of asthma with higher sensitivity versus a ≥ 20% decrease in FEV_1 during MCT (93.2% vs 54.9%), lower specificity (35.4% vs 85%), and higher diagnostic odds ratio (7.5% vs 6.9%).[10] Similar to

Table 5. Optimum Cutoff Values of Change in Airway Resistance Indices for the Detection of Air-Flow Limitation That Resulted in a ≥ 20% Decrease in FEV_1 During Methacholine Challenge Testing

Airway Resistance Parameter*	Area Under the Receiver Operating Characteristic Curve	Optimum Cutoff Value	Youden Index	Sensitivity, %	Specificity, %
R_{FOT_15}	0.84	≥14.5% increase	0.59	100	59.2
R_{FOT_10}	0.82	≥21.9% increase	0.46	60	86.1
R_{aw}	0.82	≥21.9% increase	0.55	100	54.4
G_{aw}	0.82	≥17.3% decrease	0.54	100	53.8
sR_{aw}	0.82	≥77.6% increase	0.54	68	86
sG_{aw}	0.83	≥38.5% decrease	0.56	76	79.8
R_{INT}	0.85	≥4.8% increase	0.60	76	83.9

* Only airway resistance parameters for which the area under the receiver operating characteristic curve was > 0.8 are included. R_{aw} = airway resistance; R_{FOT_15} = forced oscillation resistance at 15 Hz; R_{FOT_10} = forced oscillation resistance at 10 Hz; sG_{aw} = specific airway conductance; G_{aw} = airway conductance; sR_{aw} = specific airway resistance; R_{INT} = interrupter resistance.

our data, Parker and McCool[12] observed that the mean decrease in sG_{aw} at the end of MCT in the subjects with a ≥ 20% decrease in FEV_1 was 48.4%.

There are sparse published data that relate to the use of the interrupter technique in MCT. Koopman et al[13] showed that air-flow limitation, demonstrated by a ≥ 20% decrease in FEV_1, corresponded to a ≥ 32.1% increase in R_{INT} but with a sensitivity and specificity of only 50% and 43%, respectively. Furthermore, a significant correlation between doses of methacholine that induced a 20% decrease in FEV_1 and 100% increase in R_{INT} ($r = 0.76$) was demonstrated by Panagou et al.[14]

The clinical importance of the excessive change in airway resistance parameters and the absence of a ≥ 20% decrease in FEV_1 during MCT still need to be clarified. Khalid et al[38] observed that a maximum decrease in FEV_1 of 10%–20% from baseline during MCT indicates an increased risk for asthma development. However, in a 3-year observation, they found no relationship between isolated sG_{aw} decreases during MCT in subjects without a 20% FEV_1 decrease and the risk of asthma development.[38]

As expected, we observed significantly higher pre-test FeNO in the R group compared with the NR group. It was in line with the findings by Schleich et al[39] and Pedrosa et al,[40] who reported significantly higher values of FeNO among patients with respiratory symptoms, normal pre-test FEV_1, and no bronchial reversibility, and who tested positive for AHR compared with those with negative MCT results. However, Giovannini et al[41] did not observe a significant difference between pre-test FeNO in the subjects with symptoms and with normal baseline spirometry who tested positive for AHR compared with those with normal airway responsiveness.

Clinically, it is observed that a substantial proportion of methacholine FEV_1 non-responders experience asthma-like symptoms during MCT. We observed ≥ 1 respiratory symptom (cough, dyspnea, wheezing, or chest tightness) after inhalation of methacholine in 76.5% of the subjects from the NR group. The issue of methacholine FEV_1 non-responders who are symptomatic was also addressed by Bohadana et al,[42] who recorded respiratory symptoms in 38.1% of the subjects in whom methacholine did not induced a ≥ 20% fall in FEV_1. They observed greater responsiveness to methacholine expressed as a dose-response slope and a greater proportion of physician-diagnosed asthma in FEV_1 non-responders who were symptomatic compared with the subjects who were asymptomatic.[42] Similar to our data, prominent changes in sR_{aw} and R_{FOT} related to the occurrence of respiratory symptoms during a negative MCT result were recorded by Mansur et al. and van Nederveen-Bendien et al.[21,43] Furthermore, hyperinflation and gas trapping were previously reported to be associated with cough during MCT.[44] The above observation suggested that respiratory symptoms induced by methacholine in patients who did not meet the diagnostic AHR criteria might be related to small-airways obstruction.

To establish the value of airway resistance measurements for the diagnosis of AHR in clinical practice, the following questions need to be answered in future studies, with different commercial devices: which airway resistance index, or combination of indices, is the most accurate and reproducible; what is the optimum cutoff value for the change of the selected airway resistance indices to be used for the calculation of the methacholine provocative dose or concentration; what is the clinical meaning of the AHR diagnosed solely on the basis of excessive airway resistance increase without a concomitant ≥ 20% decrease in FEV_1.

This study had some limitations. First, we recruited a relatively small number of subjects and the pulmonary function measurements with the use of different techniques were performed in a non-randomized sequence. Second, the median (IQR) time interval between successive methacholine concentrations (9 [9-10] min) and between methacholine inhalation and the onset of FEV_1 measurement (5 [4–5] min) was longer than recommended in the guidelines,[3,4] which probably affected the partial cumulative

effect of inhaled methacholine. However, intervals between methacholine inhalations and between methacholine inhalation and spirometry were much lower than the reported plateau of methacholine action (mean ± SD of 74.6 ± 53.7 min[45]). Third, the main body of data was recorded before the publication of new MCT guidelines,[3] which state that MCT results should be expressed as the provocative dose that induces a 20% decrease in FEV_1.[4] Thus, the results of the MCT in our data were interpreted according to the provocative concentration of methacholine causing a 20% decrease in FEV_1.

Furthermore, symptoms during MCT were assessed only qualitatively, and no quantitative symptoms scoring system was used. Thus, 5 of 34 subjects with symptoms (14.7%) who reported respiratory symptoms during baseline pulmonary function testing were excluded from the assessment of changes in pulmonary function indices at the onset of symptoms during MCT. Finally, we decided to compare changes in the airway resistance indices between the groups dichotomized according to traditional spirometry-based MCT results. This approach did not allow us to investigate the relative sensitivity and specificity of airway resistance measurements versus FEV_1 measurement during MCT in the diagnosis of asthma. However, independent of the MCT, there is no objective reference standard for the diagnosis of asthma in patients without air-flow limitation detected by baseline spirometry.

Conclusions

The decrease in X_{FOT_10}, X_{FOT_15}, and G_{aw} as well as the increase in R_{FOT_10} and R_{aw} were significantly greater in the subjects with AHR, defined as a ≥ 20% decrease in FEV_1 during MCT compared with those with normal airway responsiveness. The airway resistance and conductance parameters measured by FOT, body plethysmography, and the interrupter technique differed significantly between NSS and the final step of the MCT both in the subjects with AHR and those with normal airway responsiveness, whereas X_{FOT_10} and X_{FOT_15} differed significantly only in the subjects with AHR. Pulmonary function parameters measured by FOT, body plethysmography, and interrupter technique are of acceptable diagnostic performance in identifying air-flow limitation, which results in a ≥ 20% decrease in FEV_1. Significant changes in airway mechanics during MCT are detectable by airway resistance measurement in FEV_1 nonresponders with methacholine-induced asthma-like symptoms. Measurement of airway resistance parameters could possibly be used as an easier-to-perform, complementary, or alternative method to spirometry in airway challenges.

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