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Methacholine challenge in young children as evaluated by spirometry and impulse oscillometry

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Summary

Background: In young children, it is difficult to obtain a reproducible flow-volume curve throughout all stages of bronchial challenge. The forced oscillation technique (FOT) is especially established in paediatrics because this method does not require forced or maximal manoeuvres and is less cooperation-dependent than conventional spirometry.

Objectives: To evaluate the association of spirometric and impulse oscillometric (IOS) indices in a short protocol for methacholine provocation.

Methods: The primary endpoint was the methacholine dose that caused a 20% decrease in FEV_1 ($PD_{-20}FEV_1$) compared to baseline. Changes in respiratory resistance (Rrs5) and reactance (Xrs5) acquired by IOS were compared with FEV_1.

Results: Forty-eight children (5.3 ± 0.9 years) were challenged. The mean maximal reduction in FEV_1 was $29.8\% \pm 14.7$ ($p < 0.0001$), the mean increase in Rsr5 was $55.3\% \pm 31.7$, and the mean decrease in Xrs5 was 0.37 kPa s $L^{-1} \pm 0.23$ ($p < 0.001$). An increase in Rrs5 of 45.2% and a decrease in Xrs5 of 0.69 kPa s L^{-1} showed the optimal combination of sensitivity and specificity to detect a 20% reduction in FEV_1 (0.72 and 0.73; 0.80 and 0.76, respectively), and the area under the ROC curve was 0.76 and 0.81, respectively ($p < 0.005$). In 28 patients with significant changes in FEV_1 and Rsr5, the $PD_{-20}FEV_1$ was 0.48 mg methacholine ± 0.59 and the $PD_{+40}Rrs5$ was 0.28 mg methacholine ± 0.45 ($p = 0.03$).

Conclusions: A short protocol for methacholine challenge testing is feasible in young children. IOS detected 70—80% of patients who responded in spirometry. During the challenge, the Rrs5 response preceded the FEV_1 response.

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Introduction

Bronchial pharmacological provocation tests, e.g., methacholine challenge testing (MCT), in young children are usually based on ATS recommendations,[1] particularly the 2-min tidal breathing method[2] and the five-breath dosimeter method[3] (both methods were successfully adapted to preschool children). Nine studies have investigated the tidal breathing method,[4–12] and six studied the dosimeter method.[13–18] However, these studies were quite variable regarding the primary outcome parameter, duration of inhalation, delivered volume, number of inhalations, and the increase in concentration between steps.

New commercially available dosimeter units in combination with safe short protocols are emerging and offer a promising alternative to ATS recommended protocols. The Aerosol Provocation System (APS with MedicAid sidestream nebuliser, CareFusion, Hoechberg, Germany) combines the advantages of the tidal breathing and five-breath protocols. In recently published papers, Merget et al.[19] and our group[20] have shown the practical use of the APS in young adults. In comparison to the five-breath dosimeter method, the cumulative dose of 1 mg methacholine was equal to a concentration of 8 mg/mL methacholine as the usually accepted cut-off point of bronchial hyperresponsiveness (BHR).[20] In a former study, Hagmolen of Have et al.[21] showed the repeatability of the APS method in 22 children aged 7–14 years. Two consecutive methacholine challenge tests revealed good agreements due to a strong intraclass correlation (ICC 0.91) and good repeatability, as measured by the method of Bland and Altman.

The sensitivity of the MCT depends on the investigated population. The most common clinical indication is to evaluate the likelihood of asthma in patients in whom the diagnosis is suggested by current symptoms but is not obvious. From an epidemiological standpoint, the probability of asthma is equal to the prevalence of asthma. When a patient presents with symptoms of asthma the probability is much higher, e.g., between 30 and 70%. Depending on the degree of the bronchial hyperresponsiveness, the post MCT likelihood of asthma might be roughly 90–98%.[1] In 135 children and young adults with asthma the sensitivity of MCT in detecting bronchial hyperresponsiveness (at or below 8 mg/mL) was 98% and methacholine was a better discriminating challenge between severity groups than the adenosine monophosphate or exercise challenges.[22]

It is difficult to obtain a reproducible flow-volume curve throughout all stages of bronchial challenge in young children, and there is an ongoing debate about the best outcome parameter.[23] The impulse oscillation system (IOS) (CareFusion, Hoechberg, Germany) and forced oscillation technique (FOT) do not require patients' efforts and may be useful in testing patients who cannot perform acceptable spirometry manoeuvres. The correlation between a 20% decrease in FEV_1 and changes in Rrs has shown that the threshold for a positive test is greater than a 35–40% increase in resistance. An increase in Rrs of less than 35–40% of baseline at the highest dose applied can be considered a negative test.[23] However, the IOS is different from the classical FOT because an impulse (a rectangular wave form) rather than a pseudorandom noise signal (a mixture of several sinusoidal wave forms) is applied by the loudspeaker, and because of differences in data processing. Especially at lower frequencies Rrs IOS was slightly greater than Rrs FOT, whereas Xrs IOS and Xrs FOT were very similar.[24]

In the present study, we investigated a short protocol for MCT in young children. After initial pulmonary function testing, children were challenged with incremental doses of methacholine. The primary endpoint was the dose that caused a 20% reduction in FEV_1 compared to baseline ($PD_{-20}FEV_1$). We compared the results of the reduction in FEV_1 with the airway resistance (Rrs5) and reactance (Xrs5) at a 5-Hz oscillatory frequency.

Materials and methods

Subjects

Children aged 3–6 years attending the outpatient clinic were recruited into the study. All children had a history of recurrent wheezing, at least two episodes with coughing that lasted longer than six weeks, and a diagnosis of recurrent bronchitis, viral-induced wheezing, or allergic asthma. Short-acting beta-agonists were withdrawn 8 h, long-acting beta-agonists 48 h, leukotriene receptor antagonists 24 h, and inhaled corticosteroids 7 days before MCT. The subjects' baseline characteristics are summarised in Table 1. The study was approved by the Ethics Committee of Goethe-University, and informed consent was obtained from the participants' parents prior to the start of the study.

Study design

This was an unblinded, cross-sectional study consisting of one visit. For baseline measurements first impulse oscillometry and after that spirometry (MasterScreen, Care-Fusion, Germany) measurements were repeated up to five times, and three manoeuvres performed with each method were recorded. Children were to produce at least three acceptable IOS measurements and three acceptable flow volume loops (FVL), with the second highest FVC and FEV_1 being within 0.1 L or 10% of the highest value.[23]

Table 1 Patient's characteristics.

		Total
Patients	[n]	48
Male/Female	[n]	29/19
Age	[yrs, mean ± SD]	5.3 ± 0.9
Weight	[kg, mean ± SD]	21.5 ± 4.2
Height	[cm, mean ± SD]	114.9 ± 7.9
BMI	[kg (m^2)$^{-1}$, mean ± SD]	16.2 ± 1.9
FVC	[% pred. mean ± SD]	91.2 ± 16.3
z-scores FVC	male [mean ± SD]	−0.08 ± 2.96
	female [mean ± SD]	−0.35 ± 1.3
FEV_1	[% pred. mean ± SD]	107.7 ± 20.0
z-scores FEV_1	male [mean ± SD]	1.15 ± 2.89
	female [mean ± SD]	0.57 ± 1.28

Consecutive MCT was performed in children with successful cooperation in pulmonary function. At each inhalation step a single oscillometry and spirometry was performed. After the challenge procedure, patients received one to three puffs of Salbutamol (0.1 mg) until the FEV_1 returned to at least 90% of the baseline value.

Forced oscillation technique

The FOT is especially established in paediatrics because this method does not require forced or maximal manoeuvres and is less cooperation-dependent than conventional spirometry. One of the latest oscillometric technologies is the Impulse Oscillometry System (IOS, CareFusion, Hoechberg, Germany).

During tidal breathing, the airflow of the subject under investigation was superimposed by small artificial pressure impulses produced by a loudspeaker generator. The advantage of impulse test signals is the relatively large spectral contents, which facilitate a thorough differentiation of pulmonary function.

The flow response of the respiratory system to these pressure impulses was recorded and analysed, providing data on the resistance of mainly conducting airways and the reactance representing the peripheral structures of the lung-thorax-system. From a recording of approximately 20 s, the average values of airway resistance Rrs5 and lung reactance Xrs5 at a 5-Hz signal frequency could be derived.

Changes in Rrs values but not in Xrs values can be expressed as percentage of baseline values. Xrs values may be very close to zero and expression of changes in X-values as a percentage would result in unrealistic numbers. For this reason, only Rsr values are helpful to quantify relative changes in individual subjects.[25] All calculations regarding the Xrs values, e.g., the ROC analysis, were made with absolute numbers.

Methacholine challenge

MCT was performed as described previously.[20] The APS dosimeter technique (CareFusion, Hoechberg, Germany) allows the computer-controlled production of aerosol using a jet-type nebuliser (Sidestream, MedicAid) powered by compressed air. The integrated pressure calibration procedure of the compressor ensured highly constant and reproducible nebuliser output. APS was calibrated to produce a continuous nebuliser output of 240 mg/min.

In children, instead of impulse nebulisation, a continuous nebulisation over the entire inspiratory cycle was preferred. Subjects were to breathe gently without holding their breath, avoidance on deep inhalations and maintain maximal inspiratory flows below 0.5 L/s. During tidal breathing, the system exactly and automatically determined the administered dose of methacholine. The system measured the effective nebulisation time and determined the cumulative dose of the required inspiratory breaths. In continuous nebulisation mode, the inhalation time and number of breaths were dependent upon the concentration of the employed methacholine and ventilation of the child. If the final cumulative dose was achieved, the nebuliser stopped immediately. Simultaneously, the flow recording was shown on screen, visualising the flow threshold to the patient.

The incremental protocol was slightly modified. The doses of inhaled methacholine with a concentration of 16 mg/mL were increased according to the following specifications (from step 1—4): 0.1, 0.4, 0.8, and 1.6 mg. Thus, the entire protocol delivered cumulative doses of 0.1, 0.5, 1.3 and 2.9 mg. Two minutes after each inhalation, oscillometry and spirometry were measured, and the individual provocation dose (PD) that caused a 20% drop in FEV_1 ($PD_{-20}FEV_1$) was calculated by logarithmic interpolation using an integrated program. In tests with a positive response at the first step, a virtual initial dose of 0.01 mg methacholine was assumed to calculate a provocative dose.[19]

Statistical methods

Statistical analyses were performed using GraphPad Prism 5.01 (GraphPad Software, Inc.). Pulmonary function parameters and PDs of methacholine were expressed as the means and standard deviations (SDs). The intra-measurement repeatability was expressed as the coefficient of variation (CV) (SDs expressed as a percentage of the mean, i.e., $100 \times SDs/mean$). For consecutive measurements, the repeatability coefficient (CR) was calculated according to the following equation: $CR = 2.83 \times$ within-subject standard variation SD_W (SD_W = division of SDs of the difference of 2 means by the square root).

To define the sensitivity and specificity of Rsr5 and Xrs5 to detect a 20% fall in FEV_1, a receiver-operating characteristic (ROC) curve was plotted. Cut-off levels were optimised using the Youden index (sensitivity + specificity − 1), and the accuracy was measured by the area under the ROC curve. In combination of Rsr5 and Xrs5 to detect a 20% fall in FEV_1 the chi-square test was used to define the sensitivity and specificity. $PD_{-20}FEV_1$ and $PD_{+40}Rsr5$ were compared to give the cumulative dose of methacholine required to reach the PDs. To assess whether the means of two groups were significantly different, the student t-test was used. Correlations between PDs were calculated using Pearson's correlation test. Probability (p) values ≤ 0.05 were considered statistically significant.

Results

Fifty-seven patients were included in the study. Seven children were not able to perform adequate PFTs, and two did not perform adequate MCTs. Forty-eight children were successfully challenged: 29 were male and 19 female, the mean age was 5.3 ± 0.9 years, and the mean height was 114.9 ± 7.9 cm. Thirteen children were diagnosed with recurrent bronchitis, 26 with viral induced wheeze, and 9 with allergic asthma. The children were not obese as indicated by the body mess index (BMI) (Table 1). Eleven of 48 patients had a positive skin prick test; 5 were mono-sensitised against birch, grass, or mites, 4 were sensitised against two allergens (birch and grass or birch and cat), and 2 were sensitised against multiple allergens (birch, grass, mite, cat, horse, and moulds).

Basic pulmonary function and MCT

All 48 children performed the spirometric measurements with a forced expiratory time (FET) of greater than 1 s and a back-extrapolated volume (VBE) of less than 80 mL or 12.5% of FVC. The mean FVC was 91.2% $\pm$ 16.3, and the mean FEV_1 106.2% $\pm$ 20.1. In three IOS measurements, the mean, SD, and coefficient of variation (CV) for Rsr5 and Xrs5 were 0.99 kPa s L^{-1} $\pm$ 0.23, CV 22.7% and 0.40 kPa s L^{-1} $\pm$ 0.12, CV 31.0%, respectively. We compared the between-test repeatability in two consecutive IOS measurements, and the repeatability coefficient (CR) was 0.073 and 0.074 for Rrs5 and Xrs5, respectively.

In MCT, the time from the beginning to the end of MCTs was 13 min, and a median of 2 step-ups (range 1—4) was needed to achieve a significant reduction in FEV_1 (Fig. 1). In 48 children, the mean maximal reduction in FEV_1 was 29.8% $\pm$ 14.7 ($p < 0.0001$), the mean increase in Rrs5 was 55.3% $\pm$ 31.7 or 0.52 kPa s L^{-1} $\pm$ 0.30, and the mean decrease in Xrs5 was 0.37 kPa s L^{-1} $\pm$ 0.23 (all $p < 0.0001$) (Tables 2a and b). To evaluate the sensitivity and specificity of Rrs5 and Xrs5 to detect a 20% drop in FEV_1, we calculated the receiver-operator characteristic (ROC) curve (Figs. 2 and 3). An Rrs5 increase of 45.2% compared to the baseline showed the optimal combination of sensitivity and specificity (0.72 [95%CI 0.54—0.86] and 0.73 [95%CI 0.45—0.92], respectively). The area under the ROC (AUC) was 0.76 ($p < 0.005$). An Xrs5 decrease of 0.69 kPa s L^{-1} showed the optimal combination of sensitivity and specificity (0.80 [95%CI 0.52—0.96] and 0.76 [95%CI 0.58—0.89], respectively), and the AUC was 0.81 ($p < 0.001$). Thus, the combination of an increase in Rsr5 $\geq$45% and/or a decrease in Xrs5 $\geq$0.69 kPa s L^{-1} showed that 39 of 48 (81%) subjects had a significant drop in spirometry (sensitivity 0.74 [95%CI 0.67—0.84], specificity 0.76 [95%CI 0.57—0.90], positive predictive value 0.87, negative predictive value 0.57).

Forty of the children had a significant reduction of greater than 20% FEV_1, and 33 children had a significant
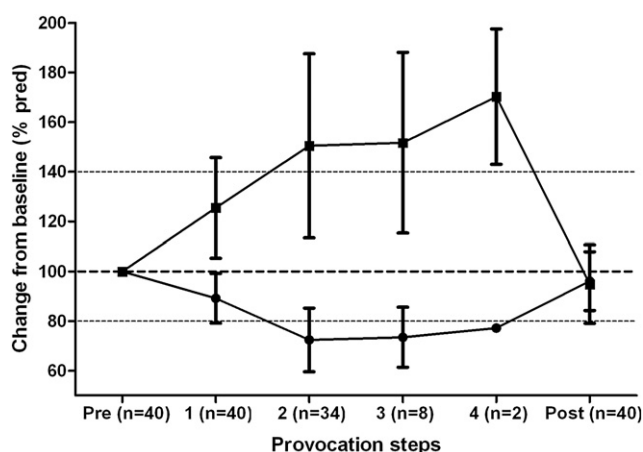

Figure 1 Changes from baseline (mean $\pm$ SD) during incremental provocation steps 1 to 4 in methacholine challenge in $n = 40$ patients with a significant fall in FEV_1. Dots indicate changes in FEV_1, and squares corresponding changes in Rrs5. "Pre" indicates baseline value of 100%; "post" indicates values after bronchodilation.

increase of greater than 40% Rsr5 as the overall accepted cut-off for a significant increase in FOT. The mean $PD_{-20}FEV_1$ was 0.42 mg $\pm$ 0.52 methacholine, and the mean $PD_{+40}Rrs5$ was 0.30 mg $\pm$ 0.43 methacholine.

We compared the PD's of 28 patients with a significant reaction in both measurements. In this group, the dose of methacholine required to achieve a significant reaction was lower in IOS compared with spirometry. The mean $PD_{-20}FEV_1$ was 0.48 mg $\pm$ 0.59 methacholine, and the mean $PD_{+40}Rrs5$ was 0.28 mg $\pm$ 0.45 methacholine ($p = 0.03$). Both parameters were significantly correlated ($r = 0.65$, $p < 0.001$) (Fig. 4). However, in the group with a cut-off of $\geq$45% Rsr5 both parameters were approaching ($n = 22$, $PD_{-20}FEV_1$ 0.37 mg $\pm$ 0.46 and $PD_{+45}Rrs5$ 0.32 mg $\pm$ 0.55 methacholine, $p = 0.39$).

Discussion

In the evaluation of MCT in a particular population (i.e., very young children), the definition of the BHR in this age group is important. However, there are unresolved issues. Current guidelines for MCT from the ERS and the ATS task forces are suitable for adults and schoolchildren but not for preschool children. Methods to assess bronchoconstriction in preschool children have been used since the 1990s, with an increasing number of studies in this age group, but the knowledge is still incomplete.[23] In a study that compared the five-breath dosimeter method with our method in young adults, a cumulative dose of 1 mg of methacholine corresponded with the concentration of 8 mg/mL methacholine as the usually accepted cut-off point of BHR.[20] Thus, reactions at lower cumulative doses than 1 mg of methacholine were considered as a positive test.

Furthermore, if a new parameter for a functional test is introduced, its distribution in the general population should be defined. Due to ethical considerations in Germany, healthy children below 14 years are not allowed to take part in MCTs to define normal values. To compare two methods and the bronchial response during MCTs, it is an advantage to examine a potentially hyperreactive population that represents the total range from severe BHR to normal BHR.

The measurement of changes in FEV_1 is still the primary outcome measure and gold standard for MCTs.[1] However, concerns exist whether spirometry is reproducible throughout all stages of MCT in young children; thus, forced oscillation techniques might be a promising alternative.[23] In a crossover study in 25 children aged 4—6 years, all children who were able to perform reproducible measurements of FEV_1 and airway resistance completed the MCT.[13] In an Australian birth cohort in children aged 5 to 7, MCT with spirometry was successfully completed in 537 of 565 (95%) of the subjects.[26] In 173 children who were aged 3, 4, 5, and 6 years, the success rate of performing MCT increased with age (55.8%, 71.6%, 88.5%, and 96%, respectively).[27] In the present study, almost all children (48 of 50, 96%) who performed reproducible basic pulmonary measurements completed the MCT.

If any technique compares with a gold standard to measure BHR, e.g., spirometry or sRaw, the results will always favour the primary objective, and another procedure will be inferior to the reference. In a comparison of

Table 2a Pulmonary function parameter total group.

		Baseline	Final step	Bronchodilator
FVC	[% pred. mean $\pm$ SD]	91.2 $\pm$ 16.3	69.3 $\pm$ 18.7	90.3 $\pm$ 19.4
FEV_1	[% pred. mean $\pm$ SD]	107.7 $\pm$ 20.0	77.9 $\pm$ 20.2	98.0 $\pm$ 12.7
Rrs5	[kPa l^{-1} s^{-1}, mean $\pm$ SD]	0.96 $\pm$ 0.23	1.48 $\pm$ 0.40	0.90 $\pm$ 0.21
Xrs5	[kPa l^{-1} s^{-1}, mean $\pm$ SD]	0.38 $\pm$ 0.14	0.75 $\pm$ 0.27	0.35 $\pm$ 0.13

Table 2b Pulmonary function parameter responder.

		Baseline	Final step	Bronchodilator
FVC	[% pred. mean $\pm$ SD]	90.8 $\pm$ 14.4	65.7 $\pm$ 14.3	86.9 $\pm$ 14.8
FEV_1	[% pred. mean $\pm$ SD]	107.2 $\pm$ 18.9	68.5 $\pm$ 8.7	96.1 $\pm$ 11.8
Rrs5	[kPa l^{-1} s^{-1}, mean $\pm$ SD]	0.98 $\pm$ 0.23	1.50 $\pm$ 0.40	1.05 $\pm$ 0.17
Xrs5	[kPa l^{-1} s^{-1}, mean $\pm$ SD]	0.42 $\pm$ 0.13	0.79 $\pm$ 0.26	0.36 $\pm$ 0.14

different lung function measurements during MCT in young children, sRaw and Xrs5 were superior to FEV_1 and Rrs5 in detecting BHR.[14] However, in this study, 48 children were screened to identify those who were capable of performing technically satisfactory and reproducible measurements, but only 25 of the children were included. Moreover, the primary objective was an increase in sRaw of more than 100%. In the study by Wilson et al.[28] in 19 asthmatic children (aged 4—11 years), PtcO2 was chosen as the reference, and only 73.4% reacted significantly in the FOT. Three of fifteen children did not develop a 40% increase in Rrs6, and no dose-response relationship was observed in any child.

Our primary objective was a decrease in FEV_1 of $\geq$20%, and the challenge was stopped if the patient achieved this cut-off. Thus, patients who might have reacted with a significant rise in Rrs5 at the next provocation step were not included. On the other hand, five patients with a non-significant decrease in FEV_1 reacted significantly in Rsr5. The relationship between both methods (spirometry and IOS) is expressed by the sensitivity and specificity as well as by the area under the roc curve (AUC). The accuracy was good to fair and confirmed the utility and the quality of the IOS to detect patients with BHR defined by the $PD_{20}FEV_1$.

Different pulmonary function parameters and different methods of unspecific bronchial challenges have a limited consistency. In the same individuals with signs and symptoms suggestive of asthma, a mannitol challenge had a sensitivity of 68% to identify MCT positive subjects with
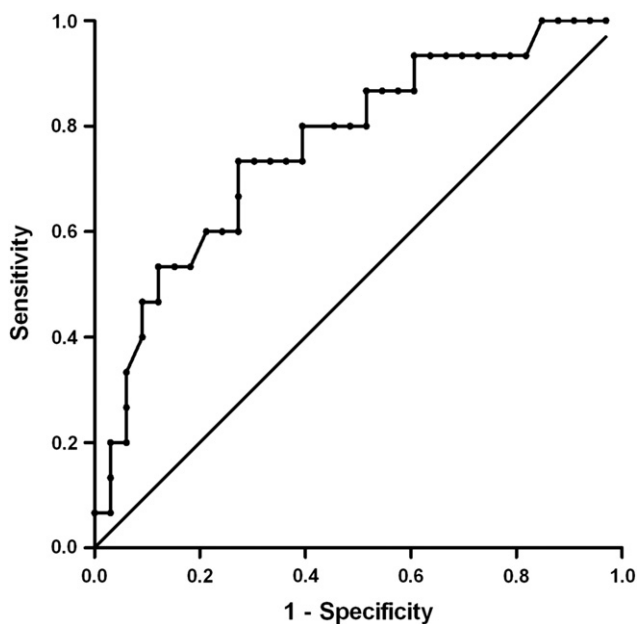

Figure 2 Receiver-operating characteristic (ROC) curve with changes of Rsr5 from baseline to detect a 20% fall in FEV_1. An increase of 45% revealed the optimum cut-off, with a sensitivity of 72% and a specificity of 73%. The area under the curve (AUC) was 0.76 ($p < 0.005$).
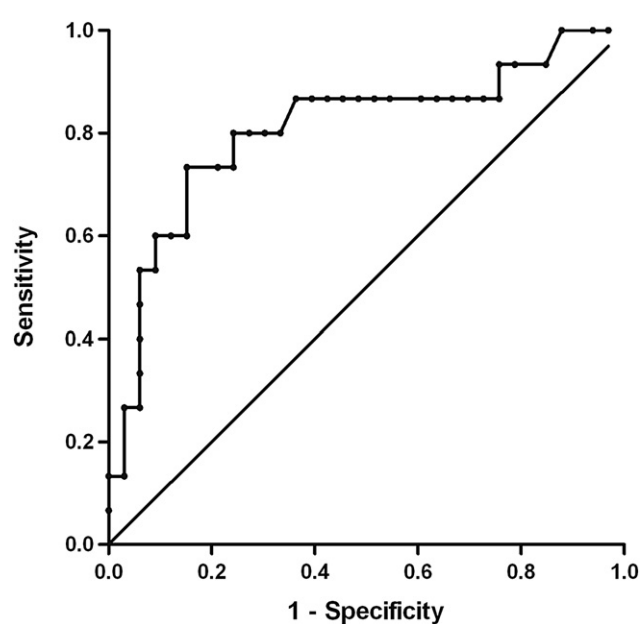

Figure 3 Receiver-operating characteristic (ROC) curve with results of Xsr5 to detect a 20% fall in FEV_1. A value of 0.69 kPa s L^{-1} Xrs5 revealed the optimum cut-off, with a sensitivity of 80% and specificity of 76%. The area under the curve (AUC) was 0.81 ($p < 0.001$).
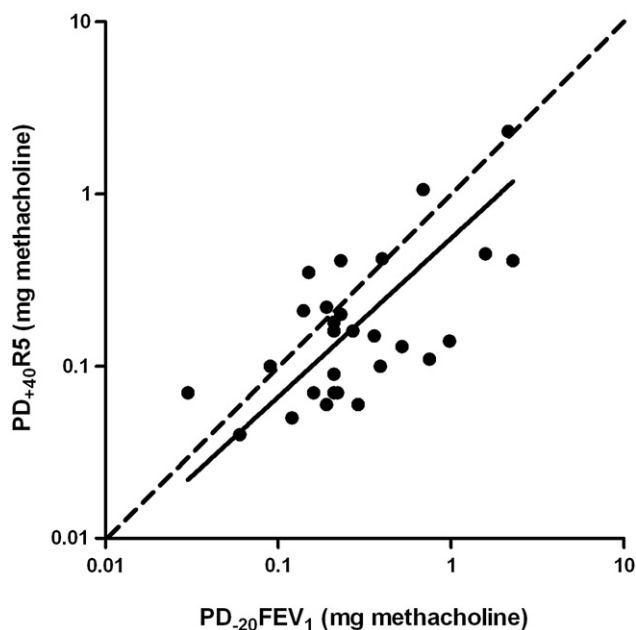
Figure 4 Log10 scale of $PD_{-20}FEV_1$ and $PD_{+40}Rrs5$ (mg methacholine) in 28 patients with a significant dual reaction. ($r = 0.65$, $p < 0.001$).

a PC_{20} of ≤ 8 mg/mL, and methacholine had a sensitivity of 62% for identifying a mannitol positive response.[29] These data suggest that different methods measure different aspects of BHR.

MCT will provide additional information to the usual examinations and recommendations in children referred to a specialised centre. Nevertheless, false negative measurements will occur. During MCT, we suggest clinically monitoring the patients by auscultation and by signs of dyspnoea. In cases of discrepancies between clinical presentation and MCT, the MCT might be postponed and repeated when the child is older and still has symptoms of asthma or BHR.

Overall, IOS and the combination of Rsr5 and Xrs5 identified young patients as having a BHR to a high degree, and the results of Xrs5 were more sensitive compared to Rsr5. These data provide more valid information about the relationship of FEV_1 and IOS in MCT.

In adults, deep inspirations before MCT protected non-asthmatics from methacholine-induced bronchoconstriction in a dose-dependent fashion but had no effect in intermittent or mild persistent asthmatics.[30] In another study, five deep breaths preceding a single dose of methacholine transiently attenuated the decreases in FEV_1 and FVC in healthy but not in mild asthmatic subjects.[31] In contrast, in asthmatics with mild airway hyperresponsiveness, deep inhalation (dosimeter) methods produced a non-significantly lower response in MCT than the standard tidal breathing method.[32] In vitro, in human bronchial segments from patients without airflow obstruction tidal oscillation had no effect on airway response to acetylcholine, whereas deep inspiration in tidally oscillating, acetylcholine-contracted airways produced potent bronchodilation.[33]

In the paediatric literature, there are few studies on the interaction of deep inspiration and bronchial reactivity. Because remodelling has not yet progressed to the same stage as in adults and dynamic changes of airway mechanics may be even more influenced by breathing patterns, the situation in children might be more interesting.[34] In children with chronic cough or asthma, when using the APS dosimeter technique and the IOS during MCT, the Rrs was significantly lower and the Xrs was significantly less negative after deep inhalations.[35] In another paediatric study,[36] which involved 4- to 7-year-old children suffering from recurrent wheezing, the effect of deep inhalations on respiratory impedance before and after MCT was measured with a forced oscillation wave-tube technique. In all children, the impedance significantly increased after MCT, and these changes were partially reversed by deep inhalation. The authors concluded that the deep inhalation is able to induce changes in airway calibre that are similar to those observed in healthy and mild asthmatics adults.

Therefore, in children, measurements with deep inhalation and forced expiration (e.g., spirometry) might produce a lower number of positive patients because children display a bronchodilation effect similar to normal or borderline adults, whereas quiet breathing parameters such as IOS/FOT lead to a higher number of positive tests while avoiding the deep inhalations of forced manoeuvres.

Moreover, the effect of deep inhalation during induced bronchoconstriction is influenced by the lung volume at which it is determined and by volume differences due to thoracic gas compression that occur during forced expiratory manoeuvres.[37] The authors concluded that measuring the maximal to partial flow ratio may result in systematic overestimation of the bronchodilator effect of deep inhalation. This error is, however, relatively small (10–12%) and may not lead to substantially erroneous conclusions. Measuring of lung volumes presupposes body plethysmography. The idea to combine spirometry, oscillometry and body plethysmography in MCTs is very interesting. However, especially in this age group, plethysmography might be difficult, and another pulmonary function measurement would extend the recommended time of approximately 5 min after methacholine inhalation and the effect of methacholine would wear off.[1,23]

Whereas changes in FEV_1 values are largely caused by changes in airflow limitation, Rrs values are more directly related to airway calibres.[25] FEV_1 is a global parameter representing central and peripheral changes in airways. Increasing peripheral obstruction is underestimated in cases in which central airway resistance is increased. Oscillometry is separated strictly between central airway resistance (Rrs) and peripheral lung reactance (Xrs).

We showed that significant increases in Rrs5 of $\geq 40\%$ preceded the reactions in FEV_1 at significantly lower doses of methacholine, which is consistent with similar findings from Vink et al.[25] In 14 of 19 children aged 5–17, the increase in resistance (especially in Rrs5) values preceded the fall in FEV_1,[25] and the authors concluded that these two parameters reflect different pathophysiological aspects of airway obstruction. In young children, Xrs IOS was equal to sRAW and was more sensitive than FEV_1 at detecting changes in lung function during MCTs. The Xrs, Rrs and sRAW but not FEV_1 revealed the subclinical bronchial obstruction prior to methacholine challenge.[14] These data suggest that apart from deep inhalation effects, oscillation techniques are more sensitive than spirometry.

Therefore, the IOS/FOT is an alternative in young children, not only because oscillation techniques are more applicable in this age group but also because these techniques might have a "methacholine-sparing" effect. However, the cumulative doses of methacholine in our protocol were low for both methods, and an Rre5 cut-off of a 45% vs. a 40% increase attenuated the advantage of IOS measurements. Interestingly, we found that in young adults, the cumulative mean dose of methacholine in our short protocol was significantly lower compared to that of the ATS-recommended five-breath dosimeter method.[20]

In 28 patients with a dual significant reaction of a decrease in FEV_1 of ≥20% and an increase in Rsr5 of ≥40%, a significant relationship between $PD_{-20}FEV_1$ and $PD_{+40}Rrs5$ was demonstrated. In FOT, Duiverman et al.[38] first described a strong correlation between $PD_{-20}FEV_1$ and $PD_{+40}Rrs6$ in asthmatic children aged 9—16 years. Wilson et al.[28] showed a significant correlation between $PD_{-20}PtcO2$ and $PD_{+40}Rrs6$ in 15 children aged 4—11 years and a significant correlation between $PC_{+35}Rrs6$ and $PC_{+15}PtcO2$ in a subgroup of 18 5-year-old children.[11] Holmgren et al.[6] defined a significant bronchial reaction to histamine as a 50% increase in Rrs FOT based on a study that included 66 children with bronchial asthma aged 8—15 years. Our findings confirm the close relationship between a classical method and the rise in airway resistance in the IOS.

In the present study, in IOS, an increase in Rrs5 of 45% and a decrease in Xrs5 of 0.69 kPa s L^{-1} revealed the optimum balance of sensitivity and specificity, detecting that 70%—80% of children had a significant reaction on spirometry. These findings are comparable with the results of Vink et al.,[25] who found an area under the ROC of 0.75 to detect a 20% drop in FEV_1 in 19 schoolchildren. Using a standardised methacholine provocation protocol, an increase in Rsr5 of 50% in IOS showed the optimal combination of sensitivity and specificity to detect a 15% reduction in FEV_1 (0.63 and 0.89, respectively). Therefore, an increase in Rrs5 IOS of 45—50% represents the threshold for detecting a positive reaction, and thresholds in Rrs5 IOS might be greater than the thresholds in Rrs6 FOT as suggested by the ATS/ERS statement.[23]

The Rrs5 IOS and the Rrs6 FOT were closely correlated ($R^2 = 0.83$), and the resistance with FOT was smaller than that with IOS with a difference that increased with decreasing frequency.[24] However, this does not necessarily mean that the IOS response at a high bronchoconstriction is unsatisfactory, particularly because the relationship between Rrs5 IOS and RAW was similar to the relationship between Rsr6 FOT and RAW ($R^2 = 0.52$ and $R^2 = 0.70$, respectively) (24.). In addition, in young children during MCTs, both methods, Rrs IOS as well as Xrs IOS, were significantly sensitive for assessing methacholine-induced changes in lung function, and both exhibited a frequency dependency with more profound changes in a lower frequency domain.[14]

The study was not designed to compare recurrent bronchitis, viral-induced wheezing, and allergic asthma. We chose these different entities because all are known to produce BHR in young children.

In conclusion, the short MCT protocol using the APS instrument in combination with a MedicAid nebuliser provides a feasible method in young children to measure BHR. Spirometry as the common pulmonary function test is practicable during bronchial challenges in this age group. Ninety-five percent of children who are able to perform reliable spirometries completed the MCT. A rise in Rsr5 of 45% and a decrease in Xrs5 of 0.69 kPa s L^{-1} showed the optimum balance between sensitivity and specificity to detect a 20% fall in FEV_1. Changes in Rsr5 slightly preceded changes in FEV_1. In young children who cannot reliably perform spirometric manoeuvres during MCT, IOS measurements are a promising alternative.

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Conflict of interest

Hans-Juergen Smith is employed at CareFusion, Hoechberg, Germany.

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