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Title: Evaluation of interrupter resistance in methacholine challenge testing in children

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33 **Running head:** R_{int} in bronchial challenge testing

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SUMMARY

Bronchial hyperresponsiveness is a key feature of asthma and is assessed using bronchial provocation tests. The primary outcome in such tests (a 20% decrease in forced expiratory volume in 1 second (FEV₁)) is difficult to measure in young patients. This study evaluated the sensitivity and specificity of the interrupter resistance (R_{int}) technique, which does not require active patient participation, by comparing it to the primary outcome measure.

Methacholine challenge tests were performed in children with a history of moderate asthma and bronchial hyperresponsiveness. Mean and individual changes in R_{int} and FEV₁ were studied. A receiver operating characteristic (ROC) curve was used to describe sensitivity and specificity of R_{int}.

Seventy-three children (median age: 9.2 yrs; range: 6.3- 13.4 yrs) participated. There was a significant (p< 0.01) increase in mean R_{int} with increasing methacholine doses. However, individual changes of R_{int} showed large fluctuations. There was great overlap in change of R_{int} between children who did and did not reach the FEV₁ endpoint. A ROC-curve showed an area under the curve of 0.65.

Because of low sensitivity and specificity, the use of R_{int} to diagnose bronchial hyperresponsiveness in individual patients seems limited.

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Key words: Bronchial hyperresponsiveness, bronchial provocation test, forced expiratory volume in 1 second, interrupter resistance, methacholine challenge test, sensitivity, specificity.

INTRODUCTION

According to GINA guidelines bronchial hyperresponsiveness (BHR) is a key feature of asthma (1). To objectify BHR, forced flow volume manoeuvres are required to measure the forced expiratory volume in one second (FEV_1), as primary outcome measure in bronchial challenge testing (2). Because of the difficulty of obtaining forced lung function measurements in early childhood the diagnosis of asthma in this age group is challenging and complicated. Several studies have advocated measurement of the interrupter resistance (R_{int}) as an alternative to quantify airway obstruction in preschool children and in children with developmental delay (3;4). The interrupter technique is non-invasive and can be obtained during tidal breathing (5). R_{int} can be measured in children from two years of age as it requires minimal cooperation (6). The R_{int} technique has been compared to body plethysmography (7) and transcutaneous partial pressure of oxygen measurements (7-10) during methacholine challenge tests, but most studies made no comparison with the outcome measure FEV_1 . The one study that compared R_{int} with FEV_1 during methacholine challenge testing reported a mean increase in R_{int} with increasing doses of methacholine (11). However, this study did not present the changes in R_{int} for individual patients nor how these related to individual changes in FEV_1 . The ability of R_{int} to detect methacholine induced bronchoconstriction in individual patients, as measured by a 20% decrease in FEV_1 , has never been studied.

The latest ATS/ERS statement on pulmonary function testing in preschool children recommended the need to determine the role of R_{int} in bronchial challenge tests (5). The current study evaluated the sensitivity and specificity of R_{int} to detect airflow obstruction during methacholine challenge tests in asthmatic children by comparing measurements with FEV_1 as the gold standard.

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MATERIALS AND METHODS

This study was a substudy of the multicentre COMBO-study in children between 6 and 16 years with moderate asthma (see online supplement) (12). Nine out of 19 hospitals performed additional R_{int} measurements during methacholine challenge tests. The children from these centres were included in this substudy. It was approved by the local medical ethical committees and written informed consent was obtained from the parents (and the child if ≥ 12 years) before start of the study. For additional information see online supplement.

Study protocol

Methacholine provocation tests were performed twice (at start of study and after 26 weeks) measuring R_{int} and FEV_1 after each methacholine dose. All children abstained from long-acting β_2 agonists and inhaled steroids for 12 hours before the first methacholine provocation test (and 36 hours before the second methacholine provocation test), and from short acting β_2 agonists for 6 hours before provocation testing. To prevent possible, unwanted physiologic effects of deep inhalation on R_{int} , R_{int} was measured prior to FEV_1 . Pulmonary function tests were performed to conform with latest ATS/ERS guidelines (2;5;13) and briefly described below. The complete methodology is described in the online supplement.

Methacholine challenge testing

Methacholine challenge tests were performed using the dosimeter method (2;14) with a nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). Methacholine chloride was administered in doubling doses and provocation was continued after at least five minutes and until the dose at which at least 20% fall in FEV_1 (PD_{20}) or the maximum dose was reached.

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109 *Interrupter resistance*

110 Airway resistance measurements were carried out using the MicroRint (Micro Medical
111 Limited, Kent, UK). A bacterial filter was used (Spirosafe bacterial filter, Micro Medical,
112 Kent, UK). Reference values of Merkus et al. (15) were used.

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114 *Spirometry*

115 Maximal expiratory flow-volume measurements were performed with standardized
116 equipment, using reference values of Stanojevic (16).

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118 *Analysis*

119 Changes in FEV_1 and R_{int} were analyzed as percentages change from baseline. Change in R_{int}
120 was also assessed as change in percentage predicted and change in z-score, computed from
121 the latest R_{int} reference values (15). Wilcoxon Signed Ranks test was used to investigate
122 whether there was a significant increase in mean change from baseline of R_{int} at each dose
123 step. To quantify the variability of FEV_1 and R_{int} within individuals between the various
124 dosesteps, coefficients of variation (CV) were calculated (see online supplement for
125 calculation). The resulting individual paired CV's of FEV_1 and R_{int} were compared using
126 Wilcoxon's test. Comparison of mean CV's between centers was done using the Kruskal-
127 Wallis test. The association between changes in FEV_1 and changes in R_{int} at PD_{20} was
128 analysed using Spearman's correlation (r_s). At each separate dose step Mann Whitney tests
129 were computed to compare the difference in changes from baseline between subjects who did
130 and who did not reach the PD_{20} endpoint at the dose step. Cox regression, with change from
131 baseline of R_{int} as time-dependent variable, was used to analyse whether these changes were
132 predictive for having reached the PD_{20} endpoint at the subsequent dose steps. The sensitivity

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and specificity of R_{int} in relation to having reached FEV₁-based PD₂₀ was described by receiver operating characteristic (ROC) curves. The area under the curve (AUC) shows whether the change in R_{int} can correctly classify those who do and do not reach the PD₂₀ endpoint. We used the coefficient of repeatability calculated by Beydon et al. (a 32.1% increase in R_{int}) as a cut-off value to calculate the sensitivity and specificity of R_{int} to predict a 20% fall in FEV₁.

For Peer Review

RESULTS

At the first COMBO-study visit 73 children with moderate asthma (60 % boys) with a median age of 9.2 years (range: 6.3 – 13.4 years) performed both R_{int} and FEV_1 measurements during methacholine challenge. Baseline characteristics are shown in Table 1. Fifty-two children reached the PD_{20} endpoint and were defined as responders (Table 2).

There was a significant increase from baseline of R_{int} starting at the sixth methacholine dose step and all dose steps thereafter (all $p < 0.01$). From the first dose step mean FEV_1 was significantly different from baseline value (all $p < 0.01$), except for dose step 2. Visual inspection of individual R_{int} profiles during the challenge test showed large fluctuations with rises and falls at subsequent dose steps. In contrast, much smoother profiles with gradual falls were found for FEV_1 (see E-figure 1 in the online supplement). To express the variability in individual curves, the CV of measured values was calculated per patient. For R_{int} the mean CV of all patients was 10.6% (10th - 90th percentile: 5.5% – 16.3%). The mean CV for FEV_1 was significantly smaller: 2.9% (1.7% - 4.3%, $p < 0.001$). Further analysis of CV R_{int} showed that there was no correlation with age ($r_s = -0.13$, $p = 0.26$). Also centres did not significantly differ from each other (Kruskal-Wallis test: $p = 0.17$).

Analysing the 52 children who reached the PD_{20} endpoint, there was no significant correlation between changes from baseline of FEV_1 and changes from baseline of R_{int} at the last dose step at which the PD_{20} endpoint was reached ($r_s = 0.11$, $p = 0.44$).

Figure 1 demonstrates the mean changes from baseline of FEV_1 and R_{int} at each dose step, while at each dose step patients are grouped according to whether or not the PD_{20} endpoint was reached at the dose step. At none of the dose steps there was a significant difference in the mean change of R_{int} from baseline between those who reached the PD_{20} endpoint at that dose step versus those who did not, except at dose step 6 ($p = 0.014$) (figure 1B). A combined analysis of all dose steps comparing responders and non-responders, using

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Cox regression analysis, showed that a greater change of R_{int} was associated with a higher probability of reaching the PD_{20} endpoint ($p= 0.038$). However, when inspecting the individual changes from baseline, there is large overlap of children who did and who did not reach the PD_{20} endpoint at the various dose steps (Figure 2). A ROC curve of changes from baseline of R_{int} as a measure to detect a 20% decrease in FEV_1 is shown in Figure 3, resulting in a small AUC of 0.65.

When using a 32.1% increase in R_{int} as cut-off value to calculate the sensitivity and specificity of R_{int} to predict a 20% fall in FEV_1 , we found a sensitivity of 50% and a specificity of 43%. This means that R_{int} was able to detect half of the responders as determined by PD_{20} and that in 12 out of 21 children R_{int} increased with more than 32% without a concomitant fall of FEV_1 with 20% .

Repeating all analyses with change in R_{int} expressed as change in percentage predicted and change in z-score led to similar results (data not shown). AUC's of ROC curves for change in percentage predicted and for change in z-score were both 0.66.

In 64 children the methacholine challenge test was repeated after 26 weeks. The analyses to compare R_{int} with FEV_1 (as described above) showed similar results (data not shown). The AUC of the ROC curve was 0.61.

DISCUSSION

This study showed that, although there was a significant increase of mean R_{int} values with higher doses of methacholine, individual changes in R_{int} are highly variable during methacholine challenge. The sensitivity and specificity of R_{int} to detect methacholine induced bronchoconstriction is low, which might limit the use of R_{int} in individual patients.

Several studies evaluated lung function measures requiring less patient effort than FEV_1 , which could possibly be used as alternative measures of changes in bronchoconstriction. Klug et al showed that R_{int} was significantly less sensitive than specific airway resistance in detecting changes in airway function (7). Phagoo et al found that R_{int} was also less sensitive and showed more variability than transcutaneous partial pressure of oxygen measurements (9;10). In a study of Beydon et al. the majority of children with a 20% fall in transcutaneous partial pressure of oxygen ($PD_{20}P_{tc}O_2$) showed a significant change of R_{int} during methacholine challenge. However, all children who did not reach the $PD_{20}P_{tc}O_2$ had at least once a significant change from baseline (8), resulting in many false positive as well as false negative outcomes.

Despite these data R_{int} is still advocated as an alternative measure for airway obstruction in those who are not able to perform forced breathing manoeuvres. Several studies showed significant correlation between gold standard FEV_1 and R_{int} in groups of patients (4;6), and reference values for R_{int} measurement in healthy controls have been published (15;17-20). Although there is convincing evidence on the value of R_{int} measurement as a measure of airway obstruction in groups of patients, the sensitivity of R_{int} to detect changes in airway obstruction in individual patients has been little researched. Only one previous study compared R_{int} with FEV_1 during methacholine challenge testing (11) and found that on average R_{int} increased during methacholine challenge comparable to changes in FEV_1 measurements, but individual changes in R_{int} were not shown. Kannisto et al. compared R_{int}

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3 211 with FEV₁ during exercise challenge to diagnose BHR (21). However, in this study only 11
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5 212 (22%) children reached the R_{int} endpoint (15% increase from baseline) and only 6 of them
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8 213 also reached the FEV₁ endpoint (10% decrease from baseline). The children who showed a
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10 214 15% rise in R_{int} but who did not have a 10% decrease in FEV₁ could be misclassified by R_{int}
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12 215 as having BHR. Davies et al. investigated the correlation between R_{int} and FEV₁ in
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17 217 patients, they found no significant correlation in individual children (22).

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20 218 To evaluate the sensitivity and specificity of the R_{int} technique to detect changes in
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22 219 airway obstruction in individual children we performed simultaneous measurements of R_{int}
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24 220 and gold standard FEV₁ in a large group of children with asthma. We show that in individual
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26 221 cases the variability of R_{int} measurements is high (mean intra-patient CV 10.6%), precluding
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28 222 proper prediction of changes in FEV₁. In the present study the ROC curve, describing the
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30 223 sensitivity and specificity of R_{int} in detecting BHR as determined by the primary outcome
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32 224 FEV₁, had a small AUC of 0.65. This predictive capacity is lower compared to impulse
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34 225 oscillometry (AUC 0.68 to 0.75 (23)).

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38 226 Large individual fluctuations in R_{int} might be due to large inpatient short-term
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40 227 variation in airway resistance during tidal breathing (11;17;20;24). This variability, resulting
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44 229 airway obstruction as required in clinical practice. Furthermore the sensitivity might be lower
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46 230 compared to FEV₁ because during (severe) airflow obstruction equilibration of alveolar
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48 231 pressure might take longer than is realised during the shutter closure time during R_{int}
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50 232 measurement (25). This may explain the lower R_{int} values in case of (severe) peripheral
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52 233 airway obstruction and hence lead to misclassifications during methacholine challenge
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56 234 testing. A third explanation of high variability of R_{int} might be the variability of upper airway
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resistance. Proper positioning of the head and neck and adequate support of the cheeks is highly warranted and might lead to intra-individual variability.

The findings of our study imply that on group level BHR can be documented, since we showed on average a significant rise in R_{int} with increasing methacholine doses. This may be useful in epidemiologic research. However, R_{int} seems to have insufficient capacity to diagnose BHR in individual patients and therefore it is of limited value in BHR measurements in clinical practice.

A major strength of our study is the fact that the methacholine challenge test was repeated after 26 weeks and resulted in a similarly low sensitivity and specificity of R_{int} , which validated the results. Beside this, the present study may have some limitations. Firstly, the study was performed in children with a mean age of 9.3 years, because preschool children are seldom able to perform FEV_1 measurements. It could be questioned if the found results are applicable to younger children. Since R_{int} is a measure of total airway resistance, an induced bronchoconstriction will lead to a relative larger increase in R_{int} in young children compared to older children, because in young children lower airways resistance contributes more to total airway resistance (26). Nevertheless, the fluctuations in R_{int} are expected to be similar as the short term repeatability of R_{int} that has been found to be independent of age (20;27). Additionally we used a 20% fall as 'gold standard' in detecting methacholine induced bronchoconstriction. When we used a 32% increase in R_{int} (the coefficient of repeatability of R_{int} measurements as described by Beydon et al.) as a cut-off value to determine BHR, there were 12 children with a significant change in R_{int} but without a 20% fall in FEV_1 . The pathophysiology of a fall in FEV_1 during a forced manoeuvre might be different from an increase of R_{int} during tidal breathing. Therefore, there is a possibility that FEV_1 might not be the gold standard for bronchial challenges especially in young children.

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~~Therefore, there could have been children with BHR, who did not have a 20% fall in FEV₁ but who did have a significant increase in R_{int}. This might have resulted in too low sensitivity because of too many false positives. However, FEV₁ is the primary outcome in methacholine challenge test, and no better ‘gold standard’ is available.~~

The current study showed on average an increase of R_{int} with subsequent methacholine doses during methacholine challenge tests and therefore it might be useful as a measure of airflow obstruction in groups of patients, e.g. in large epidemiological studies. However, we conclude that R_{int} seems to have insufficient capacity to diagnose BHR in individual patients.

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8 375 **Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step**
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10 376 **patients are grouped according to whether (closed symbols) or not (open symbols) the**
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12 377 **PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent**
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15 378 **SEM.**
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21 380 **Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to**
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23 381 **whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁)**
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25 382 **was reached. At each dose step there is large overlap in changes from baseline of R_{int}**
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34 385 **Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false**
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36 386 **positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in**
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38 387 **FEV₁ (PD₂₀). Area under the curve (AUC) = 0.65.**
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Table 1: Baseline characteristics of children with moderate asthma (n=73) who performed a methacholine provocation test with both R_{int} and FEV₁ measurement at first visit.

		Median (interquartile range)
Age (years)		9.2 (7.6 – 10.6)
Height (cm)		135.6 (128.5 – 146.0)
Sex (number (%) males)		43 (58.9%)
FEV ₁ ^a	z-score	-0.26 (-0.74 – 0.32)
	% predicted	96.8 (90.8 – 104.2)
FVC ^b	z-score	0.10 (-0.37 – 0.67)
	% predicted	101.4 (95.7 – 108.3)
FEV ₁ /FVC	z-score	-0.81 (-1.34 – -0.21)
	% predicted	94.1 (89.7 – 98.6)
R_{int} ^c	z-score	0.78 (0.05 – 1.57)
	% predicted	123.4 (107.0 – 147.2)

a) FEV₁ = Forced expiratory volume in 1 second; b) FVC = Forced expiratory volume; c) R_{int} = Interrupter resistance.

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Table 2: Number of children with moderate asthma (n = 73) who reached the PD₂₀ endpoint ^{a)} at subsequent methacholine dose steps.

Dose step	Number
1 = 0.625 µg	0
2 = 1.25 µg	0
3 = 2.5 µg	0
4 = 5.0 µg	2
5 = 10.0 µg	6
6 = 20 µg	10
7 = 40 µg	10
8 = 80 µg	10
9 = 160 µg	9
10 = 320 µg	5
No threshold	21

a) PD₂₀ endpoint = dose step at which a fall of more than 20% in FEV₁ was reached.

Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.
179x190mm (96 x 96 DPI)

Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.

173x190mm (96 x 96 DPI)

Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached. At each dose step there is large overlap in changes from baseline of R_{int} between both groups.
191x190mm (96 x 96 DPI)

Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in FEV_1 (PD_{20}). Area under the curve (AUC) = 0.65.
186x161mm (96 x 96 DPI)

ONLINE DATA SUPPLEMENT

EVALUATION OF INTERRUPTER RESISTANCE IN METHACHOLINE CHALLENGE TESTING IN CHILDREN

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COMBO-Rint research group consists of: H.G.M. Arets, N.J. van den Berg, P.L.P. Brand, E.J. Duiverman, H.J. Hendriks, J.W.C.M. Heynens, J.C. van Nierop, M. Nuysink.

Financial support: This study was supported by an unconditional grant from GlaxoSmithKline Pharma Europe.

MATERIALS AND METHODS

This study was a substudy performed in some centres participating in the multicentre COMBO-study. The COMBO-study is a blinded study comparing the clinical effects of fluticasone propionate 200 mcg BD versus salmeterol/fluticasone propionate 50/100 mcg BD on number of symptoms free days in children between 6 and 16 years of age (trial registration number NCT00197106; GSK study number SAM101667) (1). It was performed in 158 patients in 19 hospitals in the Netherlands, between June 2005 and October 2008. All children had a clinical history of moderate asthma (according to NAEPP guidelines (2)) and bronchial hyperresponsiveness (BHR) (methacholine dose at which at least 20% fall in FEV₁ (PD₂₀) ≤ 150 µg). Nine hospitals performed additional Rint measurements during methacholine challenge tests, so children from these centres were included in this comparative lung function study. The study was approved by the local medical ethical committees of the different centres and written informed consent was obtained from the parents (and also from the child if ≥12 years old) before the start of the study.

Measurements

Methacholine challenge testing

Methacholine challenge tests were performed using the dosimeter method (3;4) with a nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). After baseline R_{int} and FEV₁ measurements, 0.9% NaCl was inhaled to rule out non-specific reactions and additionally methacholine chloride was administered in doubling doses (0.625 µg, 1.25 µg, 2.5 µg, 5.0 µg, 10.0 µg, 20.0 µg, 40.0 µg, 80 µg, 160 µg, 320 µg). The procedure was performed according to the latest ATS/ERS guidelines (4) as follows: after manually triggering of the dosimeter children were instructed to inhale slowly from forced residual

capacity (FRC) to total lung capacity (TLC) and then hold their breath for 2 seconds. Two minutes after each inhalation, R_{int} and FEV_1 were measured. Provocation was continued after at least five minutes and until the PD_{20} or the maximum dose was reached.

Interrupter resistance

Airway resistance measurements were carried out using the MicroRint (Micro Medical Limited, Kent, UK) that contains a pneumotachometer, a flow interruption valve and a pressure transducer to measure mouth pressure post-occlusion. A bacterial filter was used (Spirosafe bacterial filter, Micro Medical, Kent, UK). Measurements were performed conform the latest ATS/ERS guideline (5). The child was seated and diverted to ensure quiet tidal breathing. Children were breathing through a mouth piece while the technicians supported the cheeks to reduce the change in upper airway compliance. Ten interruptions of 100 ms were made at peak expiratory flow with a random frequency, with the valve closing within 10 ms. The MicroRint software automatically rejected measurements that showed an artefact on the pressure curve. Additionally measurements that showed tachypnoea or irregular breathing, vocalisation, or hyperextension or flexion of the neck were manually discarded as well as were tracings with a horizontal or declining pressure signal suggesting air leakage around the mouthpiece. R_{int} values were calculated using the two-point linear fit back extrapolation technique to $t=15\text{ms}$ (6). A minimum of five successful interruptions was required to calculate the median R_{int} value. Reference values of Merkus et al. (7) were used to convert R_{int} outcomes into percentage predicted values.

Spirometry

Maximal expiratory flow-volume measurements were performed with standardized equipment. Seven centres used a Jaeger pneumotachometer (Viasys Healthcare, Hochberg,

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Germany), 1 centre used the Microloop (Micro Medical, Kent, UK) and 1 centre used a Zan
spirometer (nSpire Health GmbH, Oberthulba, Germany). Measurements were performed
according to the latest ATS/ERS guidelines (8) with the child sitting and wearing a nose clip,
without using a bacterial filter. Equipment calibration was performed daily and all
measurements were BTPS corrected. After a full inspiration children performed a maximally
forced and full expiration. At least three technician-accepted flow-volume curves were
obtained and the largest FEV₁ was selected. Reference values of Stanojevic (9) were used.

Analysis; Calculation of inpatient coefficients of variation (CV):

To quantify the variability of FEV₁ and R_{int} measurements between the various dose steps
during the methacholine provocation test, a coefficient of variation (CV) was calculated for
each separate patient. This was done by fitting smooth third degree polynomials of each
parameter separately versus log-methacholine dose for each individual patient to allow a
gradual change over the subsequent dose steps. The resulting standard deviation of residuals
for each patient was subsequently divided by the same patients mean value to obtain an
individual CV of the measurements. The resulting individual paired CV's of FEV₁ and R_{int}
were compared using Wilcoxon's test.

RESULTS

Visual inspection of individual R_{int} profiles during the challenge test showed large
fluctuations with rises and falls at subsequent dose steps. In contrast, much smoother profiles
with gradual falls were found for FEV₁ (E-figure 1).

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E-FIGURE LEGEND

E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline.

E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline. 190x196mm (96 x 96 DPI)

E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline. 190x199mm (96 x 96 DPI)

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Title: Evaluation of interrupter resistance in methacholine challenge testing in children

Authors: Marije Koopman, MD¹; Hein J.L. Brackel, MD, PhD²; Anja A.P.H. Vaessen-Verberne, MD, PhD³; Wim C. Hop, PhD⁴; Cornelis K. van der Ent, MD, PhD¹; on behalf of the COMBO-Rint research group.

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29 GlaxoSmithKline Pharma Europe.

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31 **This article has supplementary material.**

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33 **Running head:** R_{int} in bronchial challenge testing

SUMMARY

Bronchial hyperresponsiveness is a key feature of asthma and is assessed using bronchial provocation tests. The primary outcome in such tests (a 20% decrease in forced expiratory volume in 1 second (FEV₁)) is difficult to measure in young patients. This study evaluated the sensitivity and specificity of the interrupter resistance (R_{int}) technique, which does not require active patient participation, by comparing it to the primary outcome measure.

Methacholine challenge tests were performed in children with a history of moderate asthma and bronchial hyperresponsiveness. Mean and individual changes in R_{int} and FEV₁ were studied. A receiver operating characteristic (ROC) curve was used to describe sensitivity and specificity of R_{int}.

Seventy-three children (median age: 9.2 yrs; range: 6.3- 13.4 yrs) participated. There was a significant (p< 0.01) increase in mean R_{int} with increasing methacholine doses. However, individual changes of R_{int} showed large fluctuations. There was great overlap in change of R_{int} between children who did and did not reach the FEV₁ endpoint. A ROC-curve showed an area under the curve of 0.65.

Because of low sensitivity and specificity, the use of R_{int} to diagnose bronchial hyperresponsiveness in individual patients seems limited.

Word count abstract: 190

Key words: Bronchial hyperresponsiveness, bronchial provocation test, forced expiratory volume in 1 second, interrupter resistance, methacholine challenge test, sensitivity, specificity.

INTRODUCTION

According to GINA guidelines bronchial hyperresponsiveness (BHR) is a key feature of asthma (1). To objectify BHR, forced flow volume manoeuvres are required to measure the forced expiratory volume in one second (FEV_1), as primary outcome measure in bronchial challenge testing (2). Because of the difficulty of obtaining forced lung function measurements in early childhood the diagnosis of asthma in this age group is challenging and complicated. Several studies have advocated measurement of the interrupter resistance (R_{int}) as an alternative to quantify airway obstruction in preschool children and in children with developmental delay (3;4). The interrupter technique is non-invasive and can be obtained during tidal breathing (5). R_{int} can be measured in children from two years of age as it requires minimal cooperation (6). The R_{int} technique has been compared to body plethysmography (7) and transcutaneous partial pressure of oxygen measurements (7-10) during methacholine challenge tests, but most studies made no comparison with the outcome measure FEV_1 . The one study that compared R_{int} with FEV_1 during methacholine challenge testing reported a mean increase in R_{int} with increasing doses of methacholine (11). However, this study did not present the changes in R_{int} for individual patients nor how these related to individual changes in FEV_1 . The ability of R_{int} to detect methacholine induced bronchoconstriction in individual patients, as measured by a 20% decrease in FEV_1 , has never been studied.

The latest ATS/ERS statement on pulmonary function testing in preschool children recommended the need to determine the role of R_{int} in bronchial challenge tests (5). The current study evaluated the sensitivity and specificity of R_{int} to detect airflow obstruction during methacholine challenge tests in asthmatic children by comparing measurements with FEV_1 as the gold standard.

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MATERIALS AND METHODS

This study was a substudy of the multicentre COMBO-study in children between 6 and 16 years with moderate asthma (see online supplement) (12). Nine out of 19 hospitals performed additional R_{int} measurements during methacholine challenge tests. The children from these centres were included in this substudy. It was approved by the local medical ethical committees and written informed consent was obtained from the parents (and the child if ≥ 12 years) before start of the study. For additional information see online supplement.

Study protocol

Methacholine provocation tests were performed twice (at start of study and after 26 weeks) measuring R_{int} and FEV_1 after each methacholine dose. All children abstained from long-acting β_2 agonists and inhaled steroids for 12 hours before the first methacholine provocation test (and 36 hours before the second methacholine provocation test), and from short acting β_2 agonists for 6 hours before provocation testing. To prevent possible, unwanted physiologic effects of deep inhalation on R_{int} , R_{int} was measured prior to FEV_1 . Pulmonary function tests were performed to conform with latest ATS/ERS guidelines (2;5;13) and briefly described below. The complete methodology is described in the online supplement.

Methacholine challenge testing

Methacholine challenge tests were performed using the dosimeter method (2;14) with a nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). Methacholine chloride was administered in doubling doses and provocation was continued after at least five minutes and until the dose at which at least 20% fall in FEV_1 (PD_{20}) or the maximum dose was reached.

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109 *Interrupter resistance*

110 Airway resistance measurements were carried out using the MicroRint (Micro Medical
111 Limited, Kent, UK). A bacterial filter was used (Spirosafe bacterial filter, Micro Medical,
112 Kent, UK). Reference values of Merkus et al. (15) were used.

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114 *Spirometry*

115 Maximal expiratory flow-volume measurements were performed with standardized
116 equipment, using reference values of Stanojevic (16).

117

118 *Analysis*

119 Changes in FEV_1 and R_{int} were analyzed as percentages change from baseline. Change in R_{int}
120 was also assessed as change in percentage predicted and change in z-score, computed from
121 the latest R_{int} reference values (15). Wilcoxon Signed Ranks test was used to investigate
122 whether there was a significant increase in mean change from baseline of R_{int} at each dose
123 step. To quantify the variability of FEV_1 and R_{int} within individuals between the various
124 dosesteps, coefficients of variation (CV) were calculated (see online supplement for
125 calculation). The resulting individual paired CV's of FEV_1 and R_{int} were compared using
126 Wilcoxon's test. Comparison of mean CV's between centers was done using the Kruskal-
127 Wallis test. The association between changes in FEV_1 and changes in R_{int} at PD_{20} was
128 analysed using Spearman's correlation (r_s). At each separate dose step Mann Whitney tests
129 were computed to compare the difference in changes from baseline between subjects who did
130 and who did not reach the PD_{20} endpoint at the dose step. Cox regression, with change from
131 baseline of R_{int} as time-dependent variable, was used to analyse whether these changes were
132 predictive for having reached the PD_{20} endpoint at the subsequent dose steps. The sensitivity

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and specificity of R_{int} in relation to having reached FEV₁-based PD₂₀ was described by receiver operating characteristic (ROC) curves. The area under the curve (AUC) shows whether the change in R_{int} can correctly classify those who do and do not reach the PD₂₀ endpoint. We used the coefficient of repeatability calculated by Beydon et al. (a 32.1% increase in R_{int}) as a cut-off value to calculate the sensitivity and specificity of R_{int} to predict a 20% fall in FEV₁.

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RESULTS

At the first COMBO-study visit 73 children with moderate asthma (60 % boys) with a median age of 9.2 years (range: 6.3 – 13.4 years) performed both R_{int} and FEV_1 measurements during methacholine challenge. Baseline characteristics are shown in Table 1. Fifty-two children reached the PD_{20} endpoint and were defined as responders (Table 2).

There was a significant increase from baseline of R_{int} starting at the sixth methacholine dose step and all dose steps thereafter (all $p < 0.01$). From the first dose step mean FEV_1 was significantly different from baseline value (all $p < 0.01$), except for dose step 2. Visual inspection of individual R_{int} profiles during the challenge test showed large fluctuations with rises and falls at subsequent dose steps. In contrast, much smoother profiles with gradual falls were found for FEV_1 (see E-figure 1 in the online supplement). To express the variability in individual curves, the CV of measured values was calculated per patient. For R_{int} the mean CV of all patients was 10.6% (10th - 90th percentile: 5.5% – 16.3%). The mean CV for FEV_1 was significantly smaller: 2.9% (1.7% - 4.3%, $p < 0.001$). Further analysis of CV R_{int} showed that there was no correlation with age ($r_s = -0.13$, $p = 0.26$). Also centres did not significantly differ from each other (Kruskal-Wallis test: $p = 0.17$).

Analysing the 52 children who reached the PD_{20} endpoint, there was no significant correlation between changes from baseline of FEV_1 and changes from baseline of R_{int} at the last dose step at which the PD_{20} endpoint was reached ($r_s = 0.11$, $p = 0.44$).

Figure 1 demonstrates the mean changes from baseline of FEV_1 and R_{int} at each dose step, while at each dose step patients are grouped according to whether or not the PD_{20} endpoint was reached at the dose step. At none of the dose steps there was a significant difference in the mean change of R_{int} from baseline between those who reached the PD_{20} endpoint at that dose step versus those who did not, except at dose step 6 ($p = 0.014$) (figure 1B). A combined analysis of all dose steps comparing responders and non-responders, using

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Cox regression analysis, showed that a greater change of R_{int} was associated with a higher probability of reaching the PD₂₀ endpoint ($p= 0.038$). However, when inspecting the individual changes from baseline, there is large overlap of children who did and who did not reach the PD₂₀ endpoint at the various dose steps (Figure 2). A ROC curve of changes from baseline of R_{int} as a measure to detect a 20% decrease in FEV₁ is shown in Figure 3, resulting in a small AUC of 0.65.

When using a 32.1% increase in R_{int} as cut-off value to calculate the sensitivity and specificity of R_{int} to predict a 20% fall in FEV₁, we found a sensitivity of 50% and a specificity of 43%. This means that R_{int} was able to detect half of the responders as determined by PD₂₀ and that in 12 out of 21 children R_{int} increased with more than 32% without a concomitant fall of FEV₁ with 20% .

Repeating all analyses with change in R_{int} expressed as change in percentage predicted and change in z-score led to similar results (data not shown). AUC's of ROC curves for change in percentage predicted and for change in z-score were both 0.66.

In 64 children the methacholine challenge test was repeated after 26 weeks. The analyses to compare R_{int} with FEV₁ (as described above) showed similar results (data not shown). The AUC of the ROC curve was 0.61.

DISCUSSION

This study showed that, although there was a significant increase of mean R_{int} values with higher doses of methacholine, individual changes in R_{int} are highly variable during methacholine challenge. The sensitivity and specificity of R_{int} to detect methacholine induced bronchoconstriction is low, which might limit the use of R_{int} in individual patients.

Several studies evaluated lung function measures requiring less patient effort than FEV_1 , which could possibly be used as alternative measures of changes in bronchoconstriction. Klug et al showed that R_{int} was significantly less sensitive than specific airway resistance in detecting changes in airway function (7). Phagoo et al found that R_{int} was also less sensitive and showed more variability than transcutaneous partial pressure of oxygen measurements (9;10). In a study of Beydon et al. the majority of children with a 20% fall in transcutaneous partial pressure of oxygen ($PD_{20}P_{tc}O_2$) showed a significant change of R_{int} during methacholine challenge. However, all children who did not reach the $PD_{20}P_{tc}O_2$ had at least once a significant change from baseline (8), resulting in many false positive as well as false negative outcomes.

Despite these data R_{int} is still advocated as an alternative measure for airway obstruction in those who are not able to perform forced breathing manoeuvres. Several studies showed significant correlation between gold standard FEV_1 and R_{int} in groups of patients (4;6), and reference values for R_{int} measurement in healthy controls have been published (15;17-20). Although there is convincing evidence on the value of R_{int} measurement as a measure of airway obstruction in groups of patients, the sensitivity of R_{int} to detect changes in airway obstruction in individual patients has been little researched. Only one previous study compared R_{int} with FEV_1 during methacholine challenge testing (11) and found that on average R_{int} increased during methacholine challenge comparable to changes in FEV_1 measurements, but individual changes in R_{int} were not shown. Kannisto et al. compared R_{int}

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3 211 with FEV₁ during exercise challenge to diagnose BHR (21). However, in this study only 11
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5 212 (22%) children reached the R_{int} endpoint (15% increase from baseline) and only 6 of them
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8 213 also reached the FEV₁ endpoint (10% decrease from baseline). The children who showed a
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10 214 15% rise in R_{int} but who did not have a 10% decrease in FEV₁ could be misclassified by R_{int}
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13 215 as having BHR. Davies et al. investigated the correlation between R_{int} and FEV₁ in
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15 216 bronchodilator response, and despite a correlation between FEV₁ and R_{int} in a group of
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17 217 patients, they found no significant correlation in individual children (22).

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20 218 To evaluate the sensitivity and specificity of the R_{int} technique to detect changes in
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22 219 airway obstruction in individual children we performed simultaneous measurements of R_{int}
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25 220 and gold standard FEV₁ in a large group of children with asthma. We show that in individual
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27 221 cases the variability of R_{int} measurements is high (mean intra-patient CV 10.6%), precluding
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29 222 proper prediction of changes in FEV₁. In the present study the ROC curve, describing the
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31 223 sensitivity and specificity of R_{int} in detecting BHR as determined by the primary outcome
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34 224 FEV₁, had a small AUC of 0.65. This predictive capacity is lower compared to impulse
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36 225 oscillometry (AUC 0.68 to 0.75 (23)).

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39 226 Large individual fluctuations in R_{int} might be due to large inpatient short-term
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41 227 variation in airway resistance during tidal breathing (11;17;20;24). This variability, resulting
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43 228 in low sensitivity and specificity, could limit the ability to detect thresholds in individual
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46 229 airway obstruction as required in clinical practice. Furthermore the sensitivity might be lower
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48 230 compared to FEV₁ because during (severe) airflow obstruction equilibration of alveolar
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51 231 pressure might take longer than is realised during the shutter closure time during R_{int}
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53 232 measurement (25). This may explain the lower R_{int} values in case of (severe) peripheral
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55 233 airway obstruction and hence lead to misclassifications during methacholine challenge
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58 234 testing. A third explanation of high variability of R_{int} might be the variability of upper airway
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resistance. Proper positioning of the head and neck and adequate support of the cheeks is highly warranted and might lead to intra-individual variability.

The findings of our study imply that on group level BHR can be documented, since we showed on average a significant rise in R_{int} with increasing methacholine doses. This may be useful in epidemiologic research. However, R_{int} seems to have insufficient capacity to diagnose BHR in individual patients and therefore it is of limited value in BHR measurements in clinical practice.

A major strength of our study is the fact that the methacholine challenge test was repeated after 26 weeks and resulted in a similarly low sensitivity and specificity of R_{int} , which validated the results. Beside this, the present study may have some limitations. Firstly, the study was performed in children with a mean age of 9.3 years, because preschool children are seldom able to perform FEV_1 measurements. It could be questioned if the found results are applicable to younger children. Since R_{int} is a measure of total airway resistance, an induced bronchoconstriction will lead to a relative larger increase in R_{int} in young children compared to older children, because in young children lower airways resistance contributes more to total airway resistance (26). Nevertheless, the fluctuations in R_{int} are expected to be similar as the short term repeatability of R_{int} that has been found to be independent of age (20;27). Additionally we used a 20% fall as 'gold standard' in detecting methacholine induced bronchoconstriction. When we used a 32% increase in R_{int} (the coefficient of repeatability of R_{int} measurements as described by Beydon et al.) as a cut-off value to determine BHR, there were 12 children with a significant change in R_{int} but without a 20% fall in FEV_1 . The pathophysiology of a fall in FEV_1 during a forced manoeuvre might be different from an increase of R_{int} during tidal breathing. Therefore, there is a possibility that FEV_1 might not be the gold standard for bronchial challenges especially in young children.

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259 The current study showed on average an increase of R_{int} with subsequent methacholine
260 doses during methacholine challenge tests and therefore it might be useful as a measure of
261 airflow obstruction in groups of patients, e.g. in large epidemiological studies. However, we
262 conclude that R_{int} seems to have insufficient capacity to diagnose BHR in individual patients.
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FIGURE LEGENDS

Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.

Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached. At each dose step there is large overlap in changes from baseline of R_{int} between both groups.

Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in FEV₁ (PD₂₀). Area under the curve (AUC) = 0.65.

Table 1: Baseline characteristics of children with moderate asthma (n=73) who performed a methacholine provocation test with both R_{int} and FEV₁ measurement at first visit.

		Median (interquartile range)
Age (years)		9.2 (7.6 – 10.6)
Height (cm)		135.6 (128.5 – 146.0)
Sex (number (%) males)		43 (58.9%)
FEV ₁ ^a	z-score	-0.26 (-0.74 – 0.32)
	% predicted	96.8 (90.8 – 104.2)
FVC ^b	z-score	0.10 (-0.37 – 0.67)
	% predicted	101.4 (95.7 – 108.3)
FEV ₁ /FVC	z-score	-0.81 (-1.34 – -0.21)
	% predicted	94.1 (89.7 – 98.6)
R_{int} ^c	z-score	0.78 (0.05 – 1.57)
	% predicted	123.4 (107.0 – 147.2)

a) FEV₁ = Forced expiratory volume in 1 second; b) FVC = Forced expiratory volume; c) R_{int} = Interrupter resistance.

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Table 2: Number of children with moderate asthma (n = 73) who reached the PD₂₀ endpoint ^{a)} at subsequent methacholine dose steps.

Dose step	Number
1 = 0.625 µg	0
2 = 1.25 µg	0
3 = 2.5 µg	0
4 = 5.0 µg	2
5 = 10.0 µg	6
6 = 20 µg	10
7 = 40 µg	10
8 = 80 µg	10
9 = 160 µg	9
10 = 320 µg	5
No threshold	21

a) PD₂₀ endpoint = dose step at which a fall of more than 20% in FEV₁ was reached.

Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.
179x190mm (96 x 96 DPI)

Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.

173x190mm (96 x 96 DPI)

Figure 2. Individual changes from baseline of Rint at each dose step, divided according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached. At each dose step there is large overlap in changes from baseline of R_{int} between both groups.

191x190mm (96 x 96 DPI)

Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in FEV_1 (PD_{20}). Area under the curve (AUC) = 0.65.
186x161mm (96 x 96 DPI)

ONLINE DATA SUPPLEMENT

EVALUATION OF INTERRUPTER RESISTANCE IN METHACHOLINE CHALLENGE TESTING IN CHILDREN

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MATERIALS AND METHODS

This study was a substudy performed in some centres participating in the multicentre COMBO-study. The COMBO-study is a blinded study comparing the clinical effects of fluticasone propionate 200 mcg BD versus salmeterol/fluticasone propionate 50/100 mcg BD on number of symptoms free days in children between 6 and 16 years of age (trial registration number NCT00197106; GSK study number SAM101667) (1). It was performed in 158 patients in 19 hospitals in the Netherlands, between June 2005 and October 2008. All children had a clinical history of moderate asthma (according to NAEPP guidelines (2)) and bronchial hyperresponsiveness (BHR) (methacholine dose at which at least 20% fall in FEV₁ (PD₂₀) ≤ 150 µg). Nine hospitals performed additional R_{int} measurements during methacholine challenge tests, so children from these centres were included in this comparative lung function study. The study was approved by the local medical ethical committees of the different centres and written informed consent was obtained from the parents (and also from the child if ≥12 years old) before the start of the study.

Measurements

Methacholine challenge testing

Methacholine challenge tests were performed using the dosimeter method (3;4) with a nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). After baseline R_{int} and FEV₁ measurements, 0.9% NaCl was inhaled to rule out non-specific reactions and additionally methacholine chloride was administered in doubling doses (0.625 µg, 1.25 µg, 2.5 µg, 5.0 µg, 10.0 µg, 20.0 µg, 40.0 µg, 80 µg, 160 µg, 320 µg). The procedure was performed according to the latest ATS/ERS guidelines (4) as follows: after manually triggering of the dosimeter children were instructed to inhale slowly from forced residual

capacity (FRC) to total lung capacity (TLC) and then hold their breath for 2 seconds. Two minutes after each inhalation, R_{int} and FEV_1 were measured. Provocation was continued after at least five minutes and until the PD_{20} or the maximum dose was reached.

Interrupter resistance

Airway resistance measurements were carried out using the MicroRint (Micro Medical Limited, Kent, UK) that contains a pneumotachometer, a flow interruption valve and a pressure transducer to measure mouth pressure post-occlusion. A bacterial filter was used (Spirosafe bacterial filter, Micro Medical, Kent, UK). Measurements were performed conform the latest ATS/ERS guideline (5). The child was seated and diverted to ensure quiet tidal breathing. Children were breathing through a mouth piece while the technicians supported the cheeks to reduce the change in upper airway compliance. Ten interruptions of 100 ms were made at peak expiratory flow with a random frequency, with the valve closing within 10 ms. The MicroRint software automatically rejected measurements that showed an artefact on the pressure curve. Additionally measurements that showed tachypnoea or irregular breathing, vocalisation, or hyperextension or flexion of the neck were manually discarded as well as were tracings with a horizontal or declining pressure signal suggesting air leakage around the mouthpiece. R_{int} values were calculated using the two-point linear fit back extrapolation technique to $t=15\text{ms}$ (6). A minimum of five successful interruptions was required to calculate the median R_{int} value. Reference values of Merkus et al. (7) were used to convert R_{int} outcomes into percentage predicted values.

Spirometry

Maximal expiratory flow-volume measurements were performed with standardized equipment. Seven centres used a Jaeger pneumotachometer (Viasys Healthcare, Hochberg,

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3 73 Germany), 1 centre used the Microloop (Micro Medical, Kent, UK) and 1 centre used a Zan
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5 74 spirometer (nSpire Health GmbH, Oberthulba, Germany). Measurements were performed
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8 75 according to the latest ATS/ERS guidelines (8) with the child sitting and wearing a nose clip,
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10 76 without using a bacterial filter. Equipment calibration was performed daily and all
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12 77 measurements were BTPS corrected. After a full inspiration children performed a maximally
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14 78 forced and full expiration. At least three technician-accepted flow-volume curves were
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16 79 obtained and the largest FEV₁ was selected. Reference values of Stanojevic (9) were used.
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22 81 *Analysis; Calculation of inpatient coefficients of variation (CV):*
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24 82 To quantify the variability of FEV₁ and R_{int} measurements between the various dose steps
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26 83 during the methacholine provocation test, a coefficient of variation (CV) was calculated for
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28 84 each separate patient. This was done by fitting smooth third degree polynomials of each
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30 85 parameter separately versus log-methacholine dose for each individual patient to allow a
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32 86 gradual change over the subsequent dose steps. The resulting standard deviation of residuals
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34 87 for each patient was subsequently divided by the same patients mean value to obtain an
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36 88 individual CV of the measurements. The resulting individual paired CV's of FEV₁ and R_{int}
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38 89 were compared using Wilcoxon's test.
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46 91 **RESULTS**
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48 92 Visual inspection of individual R_{int} profiles during the challenge test showed large
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50 93 fluctuations with rises and falls at subsequent dose steps. In contrast, much smoother profiles
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52 94 with gradual falls were found for FEV₁ (E-figure 1).
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E-FIGURE LEGEND

E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline.

E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline.
190x196mm (96 x 96 DPI)

E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline. 190x199mm (96 x 96 DPI)