Evaluation of interrupter resistance in methacholine challenge testing in children

Marije Koopman, Hein J.L. Brackel, Anja A.P.H. Vaessen-Verberne, Wim Cj Hop, Cornelis K van Der Ent

Pediatric Pulmonology

Evaluation of interrupter resistance in methacholine challenge testing in children

Journal:	*Pediatric Pulmonology*
Manuscript ID:	PPUL-10-0061.R2
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	08-Aug-2010
Complete List of Authors:	Koopman, Marije; University Medical Center Utrecht, Wilhelmina Children's Hospital, Pediatric Pulmonology Brackel, Hein; Catharina Hospital, Pediatrics Vaessen-Verberne, Anja; Amphia Hospital, Pediatrics Hop, Wim CJ; Erasmus MC, Department of Epidemiology & Biostatistics van der Ent, Cornelis; University Medical Center Utrecht, Wilhelmina Children's Hospital, Pediatric Pulmonology
Keywords:	Bronchial hyperresponsiveness, bronchial provocation test, forced expiratory volume in 1 second, interrupter resistance, methacholine challenge test, sensitivity, specificity

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

Authors: Marije Koopman, MD[1]; Hein J.L. Brackel, MD, PhD[2]; Anja A.P.H. Vaessen-Verberne, MD, PhD[3]; Wim C. Hop, PhD[4]; Cornelis K. van der Ent, MD, PhD[1]; on behalf of the COMBO-Rint research group.

Institutions:

1. Department of Paediatric Pulmonology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands.
2. Department of Paediatrics, Catharina Hospital, Eindhoven, the Netherlands.
3. Department of Paediatrics, Amphia Hospital, Breda, the Netherlands
4. Department of Biostatistics, Erasmus MC – University Medical Centre Rotterdam, Rotterdam, the Netherlands

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.

Corresponding author

Ms. Marije Koopman

Wilhelmina Children's Hospital/University Medical Centre Utrecht

Department of Paediatric Pulmonology, Huispostnummer KH.01.419.0

P O Box 85090, 3508 AB Utrecht, the Netherlands

Email: M.Koopman-5@umcutrecht.nl

Telephone number: 0031-88-7553201 / Fax number: 0031-88-7554747

Word count body of manuscript: 2483

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

This article has supplementary material.

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 (FEV_1)) 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 FEV_1 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 FEV_1 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.

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 β2 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 β2 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.

Interrupter resistance

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

Spirometry

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

Analysis

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

and specificity of R_{int} in relation to having reached FEV_1-based PD_{20} 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 PD_{20} 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 FEV_1.

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% (10^{th} - 90^{th} 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

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 PD20 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}

with FEV_1 during exercise challenge to diagnose BHR (21). However, in this study only 11 (22%) children reached the R_{int} endpoint (15% increase from baseline) and only 6 of them also reached the FEV_1 endpoint (10% decrease from baseline). The children who showed a 15% rise in R_{int} but who did not have a 10% decrease in FEV_1 could be misclassified by R_{int} as having BHR. Davies et al. investigated the correlation between R_{int} and FEV_1 in bronchodilator response, and despite a correlation between FEV_1 and R_{int} in a group of patients, they found no significant correlation in individual children (22).

To evaluate the sensitivity and specificity of the R_{int} technique to detect changes in airway obstruction in individual children we performed simultaneous measurements of R_{int} and gold standard FEV_1 in a large group of children with asthma. We show that in individual cases the variability of R_{int} measurements is high (mean intra-patient CV 10.6%), precluding proper prediction of changes in FEV_1. In the present study the ROC curve, describing the sensitivity and specificity of R_{int} in detecting BHR as determined by the primary outcome FEV_1, had a small AUC of 0.65. This predictive capacity is lower compared to impulse oscillometry (AUC 0.68 to 0.75 (23)).

Large individual fluctuations in R_{int} might be due to large intrapatient short-term variation in airway resistance during tidal breathing (11;17;20;24). This variability, resulting in low sensitivity and specificity, could limit the ability to detect thresholds in individual airway obstruction as required in clinical practice. Furthermore the sensitivity might be lower compared to FEV_1 because during (severe) airflow obstruction equilibration of alveolar pressure might take longer than is realised during the shutter closure time during R_{int} measurement (25). This may explain the lower R_{int} values in case of (severe) peripheral airway obstruction and hence lead to misclassifications during methacholine challenge testing. A third explanation of high variability of R_{int} might be the variability of upper airway

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.

~~Therefore, there could have been children with BHR, who did not have a 20% fall in FEV_1 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, FEV_1 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.

ACKNOWLEDGEMENTS

The authors would like to thank the lung function technicians for the measurements and the members of the COMBO-R_{int} research group for recruiting the patients. The COMBO-R_{int} research group consists of: H.G.M. Arets (University Medical Centre Utrecht), N.J. van den Berg (Flevo Hospital Almere), P.L.P. Brand (Princess Amalia Children's Hospital Zwolle), E.J. Duiverman (University Medical Centre Groningen), H.J. Hendriks (Maastricht University Medical Centre), J.W.C.M. Heynens (Maasland Hospital Sittard), J.C. van Nierop (Academical Medical Centre Amsterdam), M. Nuysink (Juliana Children's Hospital).

HJL Brackel has received fees for consulting and for speaking from GlaxoSmithKline Pharma Europe.

REFERENCES

1. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008 January;31(1):143-78.

2. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000 January;161(1):309-29.

3. Beydon N, Pin I, Matran R, Chaussain M, Boule M, Alain B, Bellet M, Amsallem F, Alberti C, Denjean A, Gaultier C. Pulmonary function tests in preschool children with asthma. *Am J Respir Crit Care Med* 2003 September 15;168(6):640-4.

4. Black J, Baxter-Jones AD, Gordon J, Findlay AL, Helms PJ. Assessment of airway function in young children with asthma: comparison of spirometry, interrupter technique, and tidal flow by inductance plethsmography. *Pediatr Pulmonol* 2004 June;37(6):548-53.

5. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007 June 15;175(12):1304-45.

6. Arets HG, Brackel HJ, van der Ent CK. Applicability of interrupter resistance measurements using the MicroRint in daily practice. *Respir Med* 2003 April;97(4):366-74.

7. Klug B, Bisgaard H. Measurement of lung function in awake 2-4-year-old asthmatic children during methacholine challenge and acute asthma: a comparison of the impulse oscillation technique, the interrupter technique, and transcutaneous measurement of oxygen versus whole-body plethysmography. *Pediatr Pulmonol* 1996 May;21(5):290-300.

8. Beydon N, Trang-Pham H, Bernard A, Gaultier C. Measurements of resistance by the interrupter technique and of transcutaneous partial pressure of oxygen in young children during methacholine challenge. *Pediatr Pulmonol* 2001 March;31(3):238-46.

9. Phagoo SB, Wilson NM, Silverman M. Evaluation of the interrupter technique for measuring change in airway resistance in 5-year-old asthmatic children. *Pediatr Pulmonol* 1995 December;20(6):387-95.

10. Phagoo SB, Wilson NM, Silverman M. Evaluation of a new interrupter device for measuring bronchial responsiveness and the response to bronchodilator in 3 year old children. *Eur Respir J* 1996 July;9(7):1374-80.

11. Bisgaard H, Klug B. Lung function measurement in awake young children. *Eur Respir J* 1995 December;8(12):2067-75.

12. Vaessen-Verberne A.A.P.H., Berg van den N.J., Nierop van J.C., Brackel H.J.L., Gerrits G.P.J.M., Hop W.C.J., Duiverman E.J. Combination therapy salmeterol/fluticasone versus doubling dose of fluticasone in children with asthma. *Am.J.Respir.Crit Care Med.* 2010 Jul 9. [Epub ahead of print]

13. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J* 2005 August;26(2):319-38.

14. Birnie D, thoe Schwartzenberg GW, Hop WC, van Essen-Zandvliet EE, Kerrebijn KF. Does the outcome of the tidal breathing and dosimeter methods of assessing bronchial responsiveness in children with asthma depend on age? *Thorax* 1990 March;45(3):199-202.

15. Merkus PJ, Stocks J, Beydon N, Lombardi E, Jones M, McKenzie SA, Kivastik J, Arets BG, Stanojevic S. Reference ranges for interrupter resistance technique: the Asthma UK Initiative. *Eur Respir J* 2010 July;36(1):157-63.

16. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, Rosenthal M, Corey M, Lebecque P, Cole TJ. Reference ranges for spirometry across all ages: a new approach. *Am J Respir Crit Care Med* 2008 February 1;177(3):253-60.

17. Lombardi E, Sly PD, Concutelli G, Novembre E, Veneruso G, Frongia G, Bernardini R, Vierucci A. Reference values of interrupter respiratory resistance in healthy preschool white children. *Thorax* 2001 September;56(9):691-5.

18. McKenzie SA, Chan E, Dundas I, Bridge PD, Pao CS, Mylonopoulou M, Healy MJ. Airway resistance measured by the interrupter technique: normative data for 2-10 year olds of three ethnicities. *Arch Dis Child* 2002 September;87(3):248-51.

19. Merkus PJ, Arets HG, Joosten T, Siero A, Brouha M, Mijnsbergen JY, de Jongste JC, van der Ent CK. Measurements of interrupter resistance: reference values for children 3-13 yrs of age. *Eur Respir J* 2002 October;20(4):907-11.

20. Oswald-Mammosser M, Llerena C, Speich JP, Donata L, Lonsdorfer. Measurements of respiratory system resistance by the interrupter technique in healthy and asthmatic children. *Pediatr Pulmonol* 1997 August;24(2):78-85.

21. Kannisto S, Vanninen E, Remes K, Korppi M. Interrupter technique for evaluation of exercise-induced bronchospasm in children. *Pediatr Pulmonol* 1999 March;27(3):203-7.

22. Davies PL, Doull IJ, Child F. The interrupter technique to assess airway responsiveness in children with cystic fibrosis. *Pediatr Pulmonol* 2007 January;42(1):23-8.

23. Vink GR, Arets HG, van der LJ, van der Ent CK. Impulse oscillometry: a measure for airway obstruction. *Pediatr Pulmonol* 2003 March;35(3):214-9.

24. Klug B, Bisgaard H. Specific airway resistance, interrupter resistance, and respiratory impedance in healthy children aged 2-7 years. *Pediatr Pulmonol* 1998 May;25(5):322-31.

25. Bates JH, Sly PD, Kochi T, Martin JG. The effect of a proximal compliance on interrupter measurements of resistance. *Respir Physiol* 1987 December;70(3):301-12.

26. Arets HG, van der Ent CK. Measurements of airway mechanics in spontaneously breathing young children. *Paediatr Respir Rev* 2004 March;5(1):77-84.

27. Chan EY, Bridge PD, Dundas I, Pao CS, Healy MJ, McKenzie SA. Repeatability of airway resistance measurements made using the interrupter technique. *Thorax* 2003 April;58(4):344-7.

FIGURE LEGENDS

Figure 1. Mean changes from baseline of FEV_1 (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) 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 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.

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.

Table 1: Baseline characteristics of children with moderate asthma (n=73) who performed a methacholine provocation test with both R_{int} and FEV_1 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) FEV_1 = Forced expiratory volume in 1 second; b) FVC = Forced expiratory volume; c) R_{int} = Interrupter resistance.

Table 2: Number of children with moderate asthma (n = 73) who reached the PD_{20} 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) PD_{20} endpoint = dose step at which a fall of more than 20% in FEV_1 was reached.

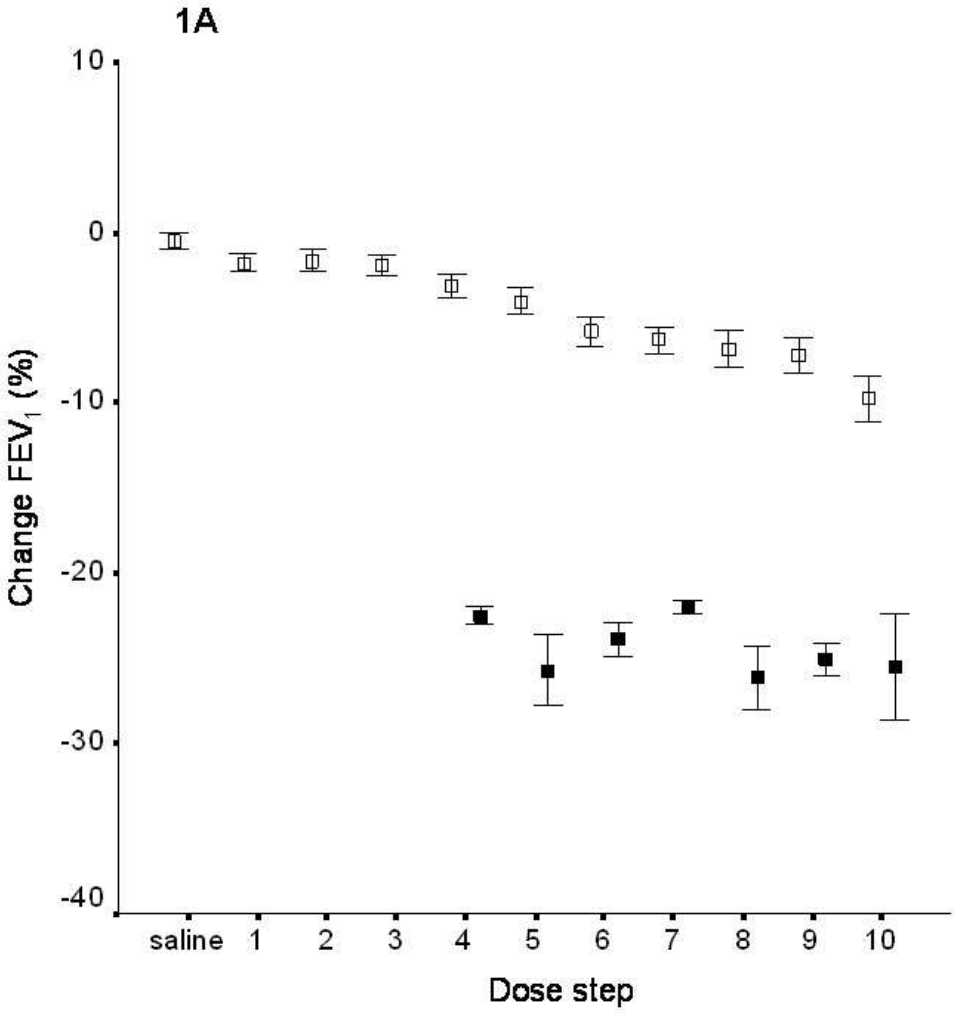


Figure 1. Mean changes from baseline of FEV_1 (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached at that dose step. Error bars represent SEM.
179x190mm (96 x 96 DPI)

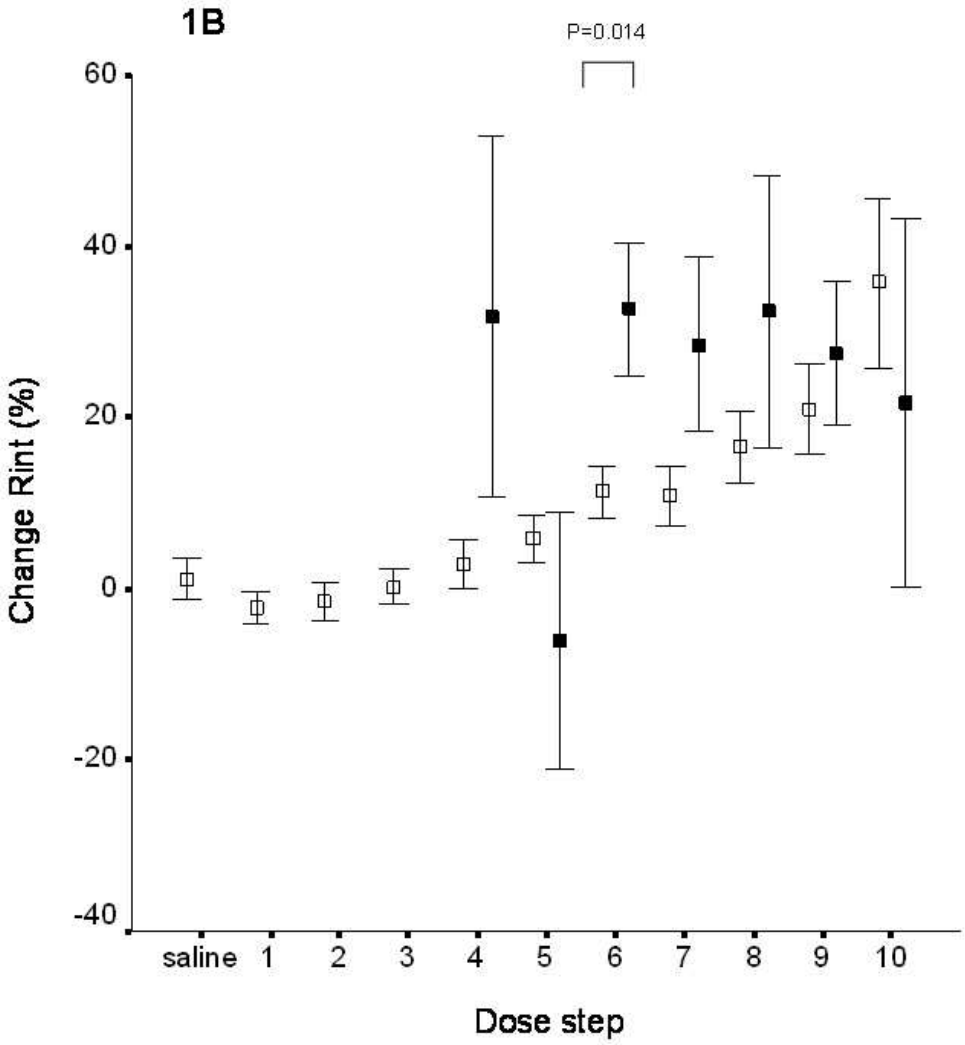


Figure 1. Mean changes from baseline of FEV_1 (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached at that dose step. Error bars represent SEM.
173x190mm (96 x 96 DPI)

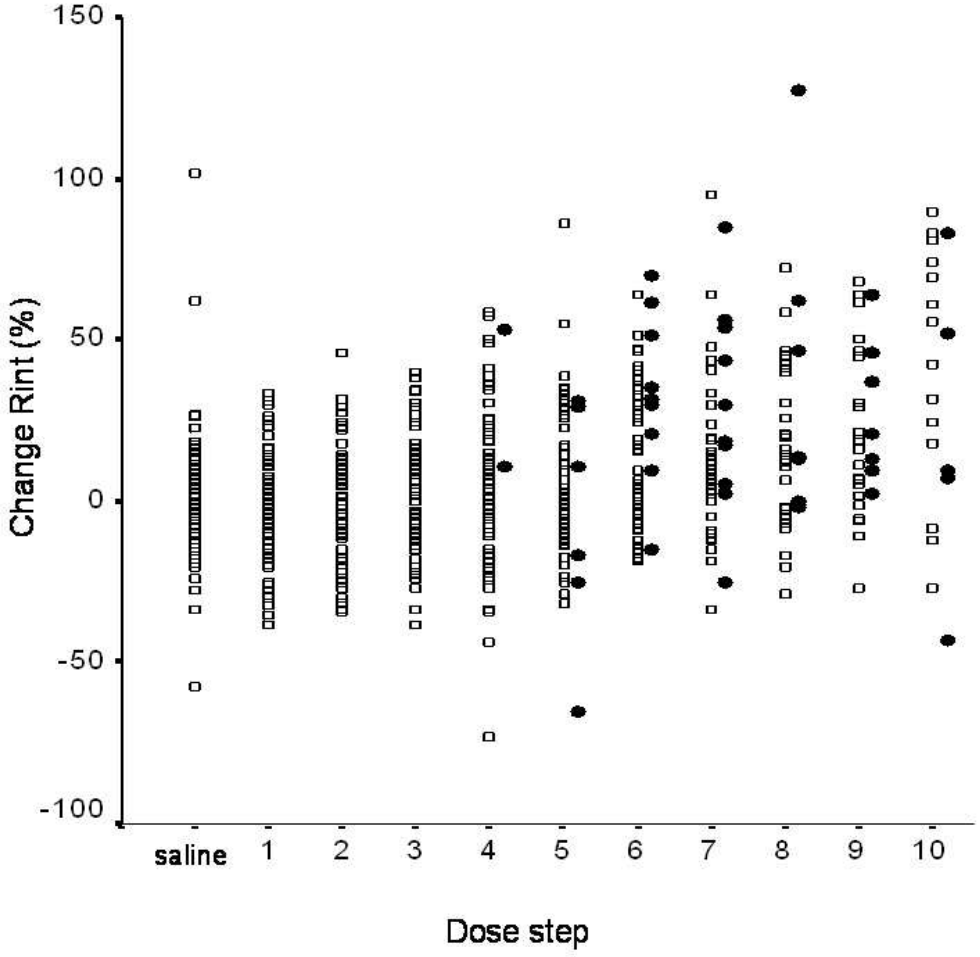


Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached. At each dose step there is large overlap in changes from baseline of R_{int} between both groups.
191x190mm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

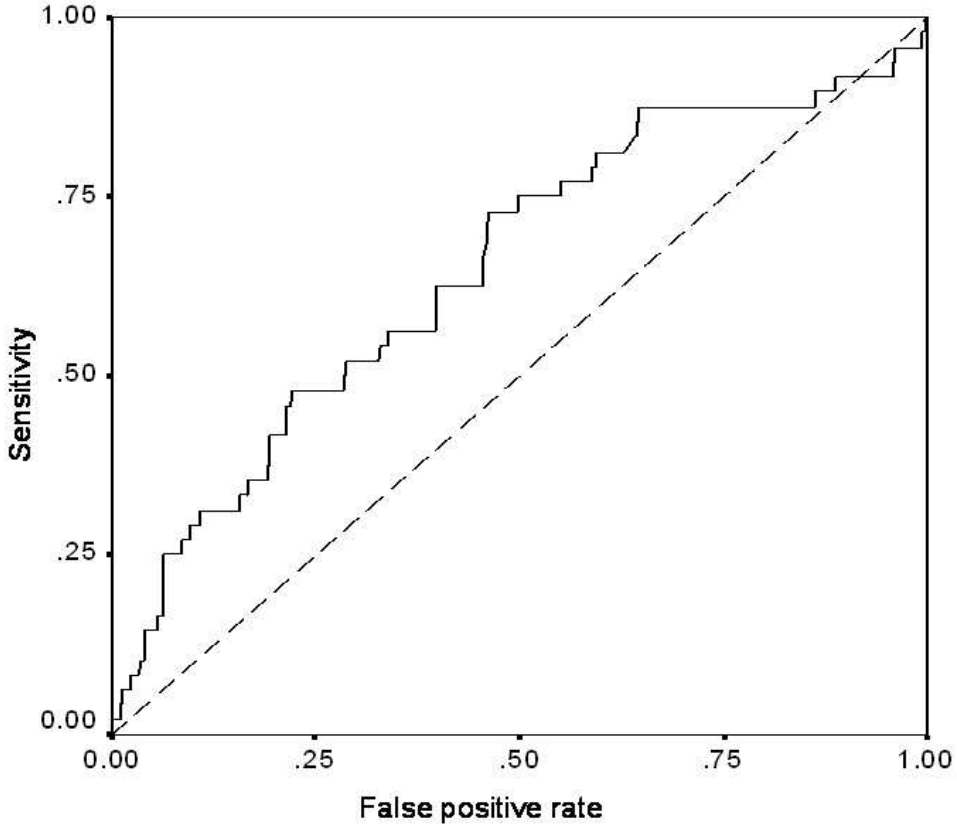


Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in FEV_1 (PD_{20}). Area under the curve (AUC) = 0.65.
186x161mm (96 x 96 DPI)

ONLINE DATA SUPPLEMENT

EVALUATION OF INTERRUPTER RESISTANCE IN METHACHOLINE CHALLENGE TESTING IN CHILDREN

Authors: Marije Koopman, MD[1]; Hein J.L. Brackel, MD, PhD[2]; Anja A.P.H. Vaessen-Verberne, MD, PhD[3]; Wim C. Hop, PhD[4]; Cornelis K. van der Ent, MD, PhD[1]; on behalf of the COMBO-Rint research group.

Institutions:

1. Department of Paediatric Pulmonology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands.
2. Department of Paediatrics, Catharina Hospital, Eindhoven, the Netherlands.
3. Department of Paediatrics, Amphia Hospital, Breda, the Netherlands
4. Department of Biostatistics, Erasmus MC – University Medical Centre Rotterdam, Rotterdam, the Netherlands

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 FEV_1 (PD_{20}) ≤ 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 FEV_1 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=15ms (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,

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 FEV_1 was selected. Reference values of Stanojevic (9) were used.

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

To quantify the variability of FEV_1 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 FEV_1 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 FEV_1 (E-figure 1).

E-REFERENCES

E1. Vaessen-Verberne A.A.P.H., Berg van den N.J., Nierop van J.C., Brackel H.J.L., Gerrits G.P.J.M., Hop W.C.J., Duiverman E.J. Combination therapy salmeterol/fluticasone versus doubling dose of fluticasone in children with asthma. *Am.J.Respir.Crit Care Med.* Accepted for publication. 2010.

E2. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. *J Allergy Clin Immunol* 2007 November;120(5 Suppl):S94-138.

E3. Birnie D, thoe Schwartzenberg GW, Hop WC, van Essen-Zandvliet EE, Kerrebijn KF. Does the outcome of the tidal breathing and dosimeter methods of assessing bronchial responsiveness in children with asthma depend on age? *Thorax* 1990 March;45(3):199-202.

E4. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000 January;161(1):309-29.

E5. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E et al. An official American Thoracic

Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007 June 15;175(12):1304-45.

E6. Phagoo SB, Wilson NM, Silverman M. Evaluation of the interrupter technique for measuring change in airway resistance in 5-year-old asthmatic children. *Pediatr Pulmonol* 1995 December;20(6):387-95.

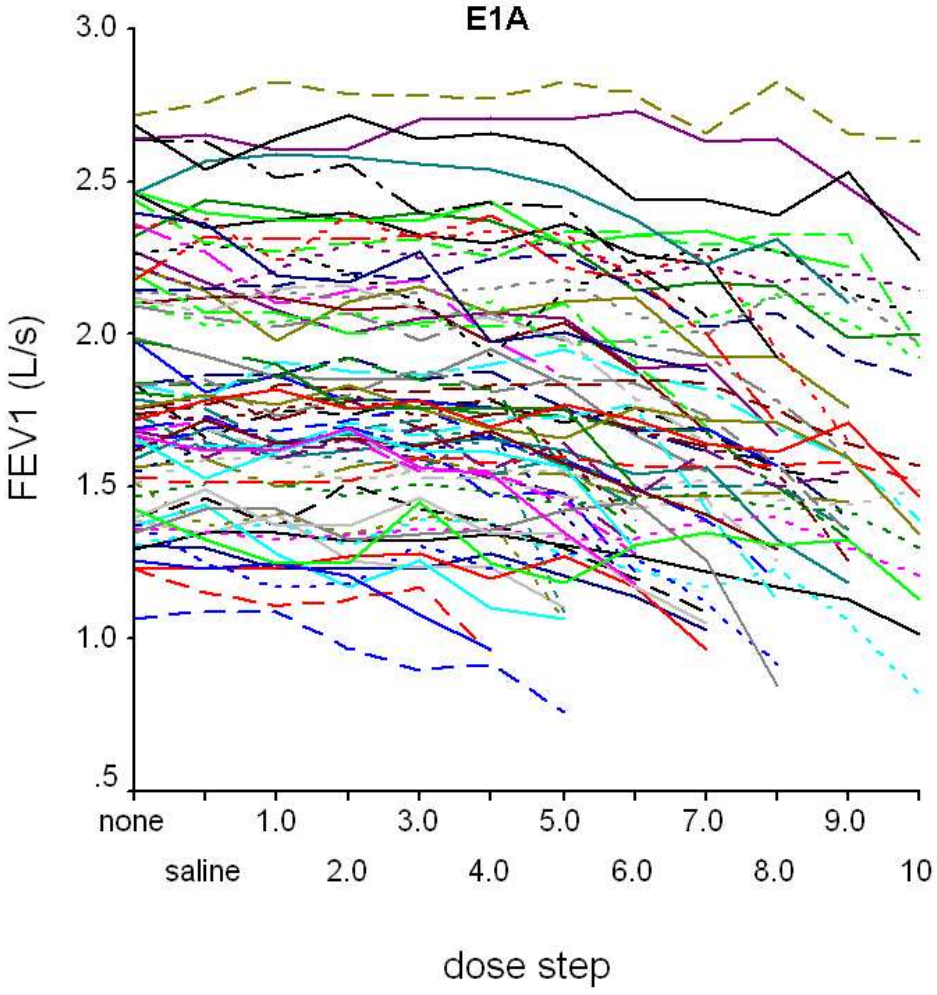
E7. Merkus PJ, Stocks J, Beydon N, Lombardi E, Jones M, McKenzie SA, Kivastik J, Arets BG, Stanojevic S. Reference ranges for interrupter resistance technique: the asthma UK initiative. *Eur Respir J* 2010 July;36(1):157-63.

E8. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J* 2005 August;26(2):319-38.

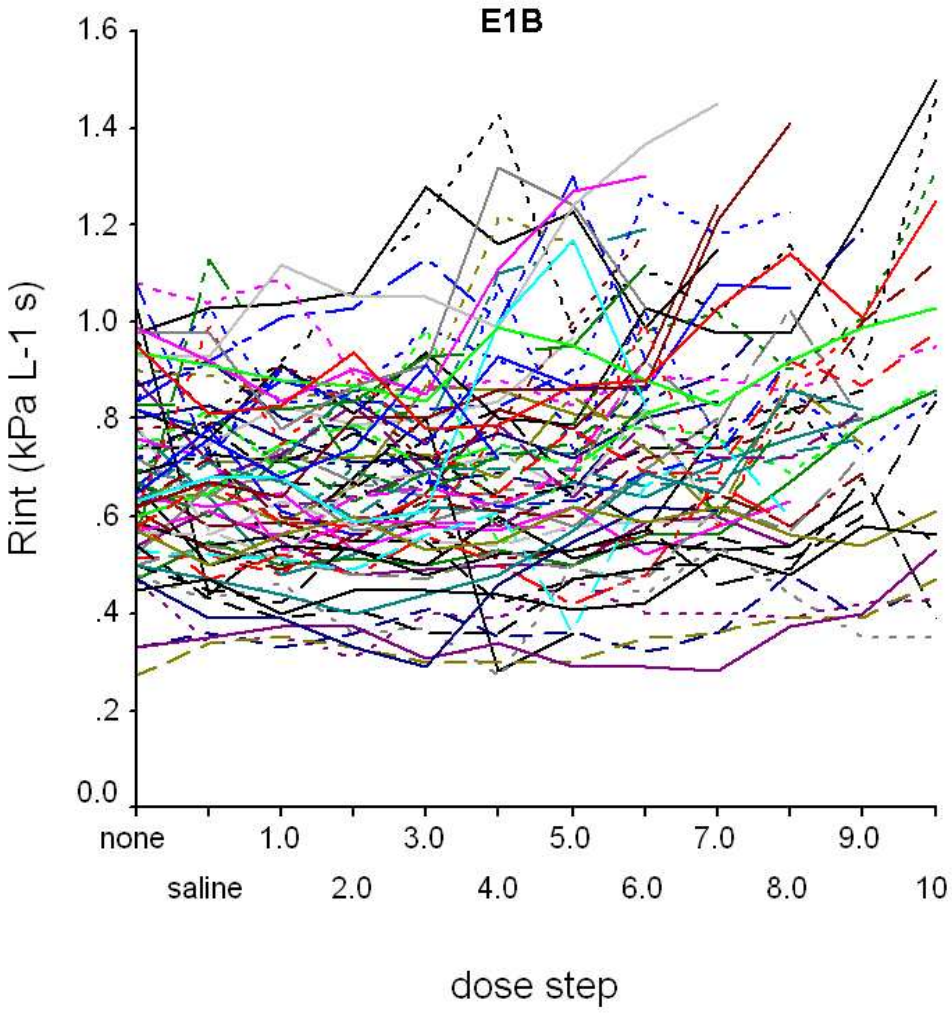
E9. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, Rosenthal M, Corey M, Lebecque P, Cole TJ. Reference ranges for spirometry across all ages: a new approach. *Am J Respir Crit Care Med* 2008 February 1;177(3):253-60.

E-FIGURE LEGEND

E-figure 1. Individual fluctuations of absolute values of FEV_1 (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 FEV_1 (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 FEV_1 (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)

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

Authors: Marije Koopman, MD[1]; Hein J.L. Brackel, MD, PhD[2]; Anja A.P.H. Vaessen-Verberne, MD, PhD[3]; Wim C. Hop, PhD[4]; Cornelis K. van der Ent, MD, PhD[1]; on behalf of the COMBO-Rint research group.

Institutions:

1. Department of Paediatric Pulmonology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands.
2. Department of Paediatrics, Catharina Hospital, Eindhoven, the Netherlands.
3. Department of Paediatrics, Amphia Hospital, Breda, the Netherlands
4. Department of Biostatistics, Erasmus MC – University Medical Centre Rotterdam, Rotterdam, the Netherlands

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.

Corresponding author

Ms. Marije Koopman

Wilhelmina Children's Hospital/University Medical Centre Utrecht

Department of Paediatric Pulmonology, Huispostnummer KH.01.419.0

P O Box 85090, 3508 AB Utrecht, the Netherlands

Email: M.Koopman-5@umcutrecht.nl

Telephone number: 0031-88-7553201 / Fax number: 0031-88-7554747

Word count body of manuscript: 2483

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

This article has supplementary material.

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 (FEV_1)) 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 FEV_1 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 FEV_1 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.

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 β2 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 β2 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.

Interrupter resistance

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

Spirometry

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

Analysis

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

and specificity of R_{int} in relation to having reached FEV_1-based PD_{20} 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 PD_{20} 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 FEV_1.

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% (10^{th} - 90^{th} 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

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 PD20 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}

with FEV_1 during exercise challenge to diagnose BHR (21). However, in this study only 11 (22%) children reached the R_{int} endpoint (15% increase from baseline) and only 6 of them also reached the FEV_1 endpoint (10% decrease from baseline). The children who showed a 15% rise in R_{int} but who did not have a 10% decrease in FEV_1 could be misclassified by R_{int} as having BHR. Davies et al. investigated the correlation between R_{int} and FEV_1 in bronchodilator response, and despite a correlation between FEV_1 and R_{int} in a group of patients, they found no significant correlation in individual children (22).

To evaluate the sensitivity and specificity of the R_{int} technique to detect changes in airway obstruction in individual children we performed simultaneous measurements of R_{int} and gold standard FEV_1 in a large group of children with asthma. We show that in individual cases the variability of R_{int} measurements is high (mean intra-patient CV 10.6%), precluding proper prediction of changes in FEV_1. In the present study the ROC curve, describing the sensitivity and specificity of R_{int} in detecting BHR as determined by the primary outcome FEV_1, had a small AUC of 0.65. This predictive capacity is lower compared to impulse oscillometry (AUC 0.68 to 0.75 (23)).

Large individual fluctuations in R_{int} might be due to large intrapatient short-term variation in airway resistance during tidal breathing (11;17;20;24). This variability, resulting in low sensitivity and specificity, could limit the ability to detect thresholds in individual airway obstruction as required in clinical practice. Furthermore the sensitivity might be lower compared to FEV_1 because during (severe) airflow obstruction equilibration of alveolar pressure might take longer than is realised during the shutter closure time during R_{int} measurement (25). This may explain the lower R_{int} values in case of (severe) peripheral airway obstruction and hence lead to misclassifications during methacholine challenge testing. A third explanation of high variability of R_{int} might be the variability of upper airway

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.

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.

ACKNOWLEDGEMENTS

The authors would like to thank the lung function technicians for the measurements and the members of the COMBO-R_{int} research group for recruiting the patients. The COMBO-R_{int} research group consists of: H.G.M. Arets (University Medical Centre Utrecht), N.J. van den Berg (Flevo Hospital Almere), P.L.P. Brand (Princess Amalia Children's Hospital Zwolle), E.J. Duiverman (University Medical Centre Groningen), H.J. Hendriks (Maastricht University Medical Centre), J.W.C.M. Heynens (Maasland Hospital Sittard), J.C. van Nierop (Academical Medical Centre Amsterdam), M. Nuysink (Juliana Children's Hospital).

HJL Brackel has received fees for consulting and for speaking from GlaxoSmithKline Pharma Europe.

REFERENCES

1. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008 January;31(1):143-78.

2. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000 January;161(1):309-29.

3. Beydon N, Pin I, Matran R, Chaussain M, Boule M, Alain B, Bellet M, Amsallem F, Alberti C, Denjean A, Gaultier C. Pulmonary function tests in preschool children with asthma. *Am J Respir Crit Care Med* 2003 September 15;168(6):640-4.

4. Black J, Baxter-Jones AD, Gordon J, Findlay AL, Helms PJ. Assessment of airway function in young children with asthma: comparison of spirometry, interrupter technique, and tidal flow by inductance plethsmography. *Pediatr Pulmonol* 2004 June;37(6):548-53.

5. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007 June 15;175(12):1304-45.

6. Arets HG, Brackel HJ, van der Ent CK. Applicability of interrupter resistance measurements using the MicroRint in daily practice. *Respir Med* 2003 April;97(4):366-74.

7. Klug B, Bisgaard H. Measurement of lung function in awake 2-4-year-old asthmatic children during methacholine challenge and acute asthma: a comparison of the impulse oscillation technique, the interrupter technique, and transcutaneous measurement of oxygen versus whole-body plethysmography. *Pediatr Pulmonol* 1996 May;21(5):290-300.

8. Beydon N, Trang-Pham H, Bernard A, Gaultier C. Measurements of resistance by the interrupter technique and of transcutaneous partial pressure of oxygen in young children during methacholine challenge. *Pediatr Pulmonol* 2001 March;31(3):238-46.

9. Phagoo SB, Wilson NM, Silverman M. Evaluation of the interrupter technique for measuring change in airway resistance in 5-year-old asthmatic children. *Pediatr Pulmonol* 1995 December;20(6):387-95.

10. Phagoo SB, Wilson NM, Silverman M. Evaluation of a new interrupter device for measuring bronchial responsiveness and the response to bronchodilator in 3 year old children. *Eur Respir J* 1996 July;9(7):1374-80.

11. Bisgaard H, Klug B. Lung function measurement in awake young children. *Eur Respir J* 1995 December;8(12):2067-75.

12. Vaessen-Verberne A.A.P.H., Berg van den N.J., Nierop van J.C., Brackel H.J.L., Gerrits G.P.J.M., Hop W.C.J., Duiverman E.J. Combination therapy salmeterol/fluticasone versus doubling dose of fluticasone in children with asthma. *Am.J.Respir.Crit Care Med.* 2010 Jul 9. [Epub ahead of print]

13. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J* 2005 August;26(2):319-38.

14. Birnie D, thoe Schwartzenberg GW, Hop WC, van Essen-Zandvliet EE, Kerrebijn KF. Does the outcome of the tidal breathing and dosimeter methods of assessing bronchial responsiveness in children with asthma depend on age? *Thorax* 1990 March;45(3):199-202.

15. Merkus PJ, Stocks J, Beydon N, Lombardi E, Jones M, McKenzie SA, Kivastik J, Arets BG, Stanojevic S. Reference ranges for interrupter resistance technique: the Asthma UK Initiative. *Eur Respir J* 2010 July;36(1):157-63.

16. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, Rosenthal M, Corey M, Lebecque P, Cole TJ. Reference ranges for spirometry across all ages: a new approach. *Am J Respir Crit Care Med* 2008 February 1;177(3):253-60.

17. Lombardi E, Sly PD, Concutelli G, Novembre E, Veneruso G, Frongia G, Bernardini R, Vierucci A. Reference values of interrupter respiratory resistance in healthy preschool white children. *Thorax* 2001 September;56(9):691-5.

18. McKenzie SA, Chan E, Dundas I, Bridge PD, Pao CS, Mylonopoulou M, Healy MJ. Airway resistance measured by the interrupter technique: normative data for 2-10 year olds of three ethnicities. *Arch Dis Child* 2002 September;87(3):248-51.

19. Merkus PJ, Arets HG, Joosten T, Siero A, Brouha M, Mijnsbergen JY, de Jongste JC, van der Ent CK. Measurements of interrupter resistance: reference values for children 3-13 yrs of age. *Eur Respir J* 2002 October;20(4):907-11.

20. Oswald-Mammosser M, Llerena C, Speich JP, Donata L, Lonsdorfer. Measurements of respiratory system resistance by the interrupter technique in healthy and asthmatic children. *Pediatr Pulmonol* 1997 August;24(2):78-85.

21. Kannisto S, Vanninen E, Remes K, Korppi M. Interrupter technique for evaluation of exercise-induced bronchospasm in children. *Pediatr Pulmonol* 1999 March;27(3):203-7.

22. Davies PL, Doull IJ, Child F. The interrupter technique to assess airway responsiveness in children with cystic fibrosis. *Pediatr Pulmonol* 2007 January;42(1):23-8.

23. Vink GR, Arets HG, van der LJ, van der Ent CK. Impulse oscillometry: a measure for airway obstruction. *Pediatr Pulmonol* 2003 March;35(3):214-9.

24. Klug B, Bisgaard H. Specific airway resistance, interrupter resistance, and respiratory impedance in healthy children aged 2-7 years. *Pediatr Pulmonol* 1998 May;25(5):322-31.

25. Bates JH, Sly PD, Kochi T, Martin JG. The effect of a proximal compliance on interrupter measurements of resistance. *Respir Physiol* 1987 December;70(3):301-12.

26. Arets HG, van der Ent CK. Measurements of airway mechanics in spontaneously breathing young children. *Paediatr Respir Rev* 2004 March;5(1):77-84.

27. Chan EY, Bridge PD, Dundas I, Pao CS, Healy MJ, McKenzie SA. Repeatability of airway resistance measurements made using the interrupter technique. *Thorax* 2003 April;58(4):344-7.

FIGURE LEGENDS

Figure 1. Mean changes from baseline of FEV_1 (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) 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 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.

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.

Table 1: Baseline characteristics of children with moderate asthma (n=73) who performed a methacholine provocation test with both R_{int} and FEV_1 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) FEV_1 = Forced expiratory volume in 1 second; b) FVC = Forced expiratory volume; c) R_{int} = Interrupter resistance.

Table 2: Number of children with moderate asthma (n = 73) who reached the PD_{20} 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) PD_{20} endpoint = dose step at which a fall of more than 20% in FEV_1 was reached.

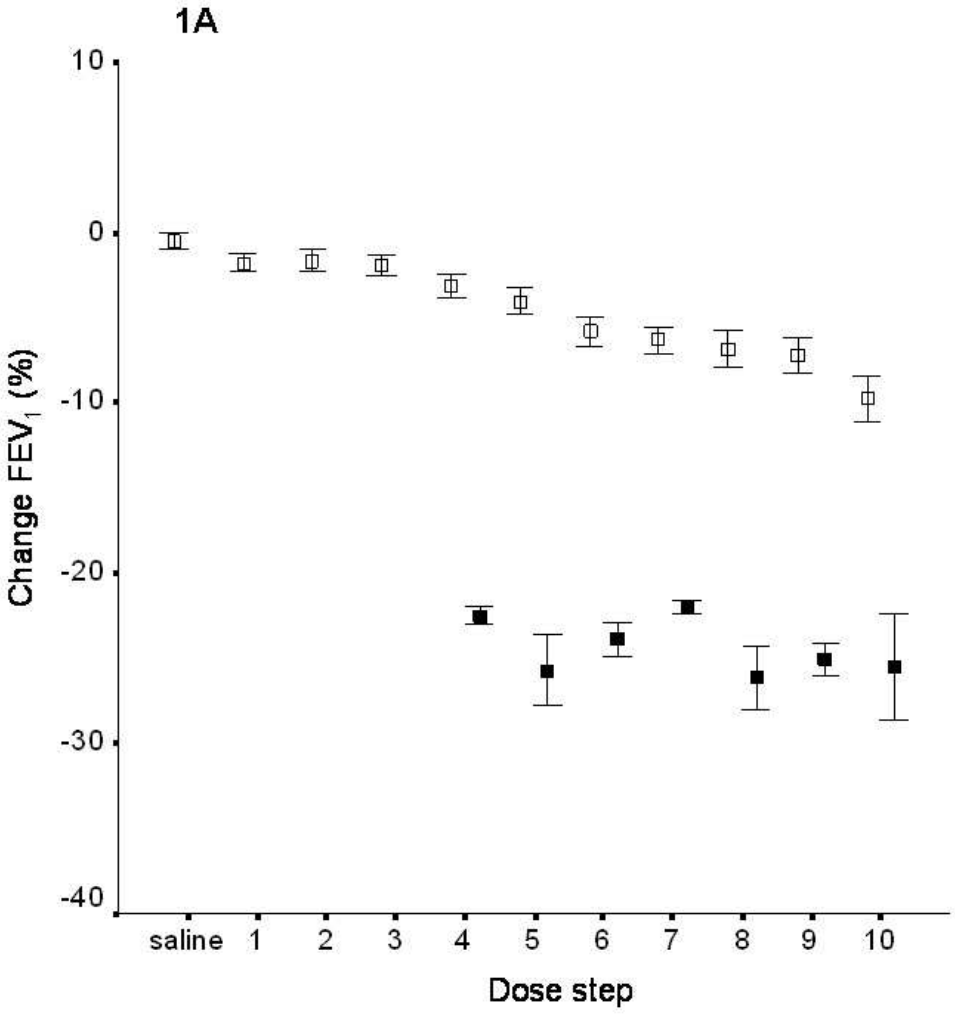


Figure 1. Mean changes from baseline of FEV_1 (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached at that dose step. Error bars represent SEM.
179x190mm (96 x 96 DPI)

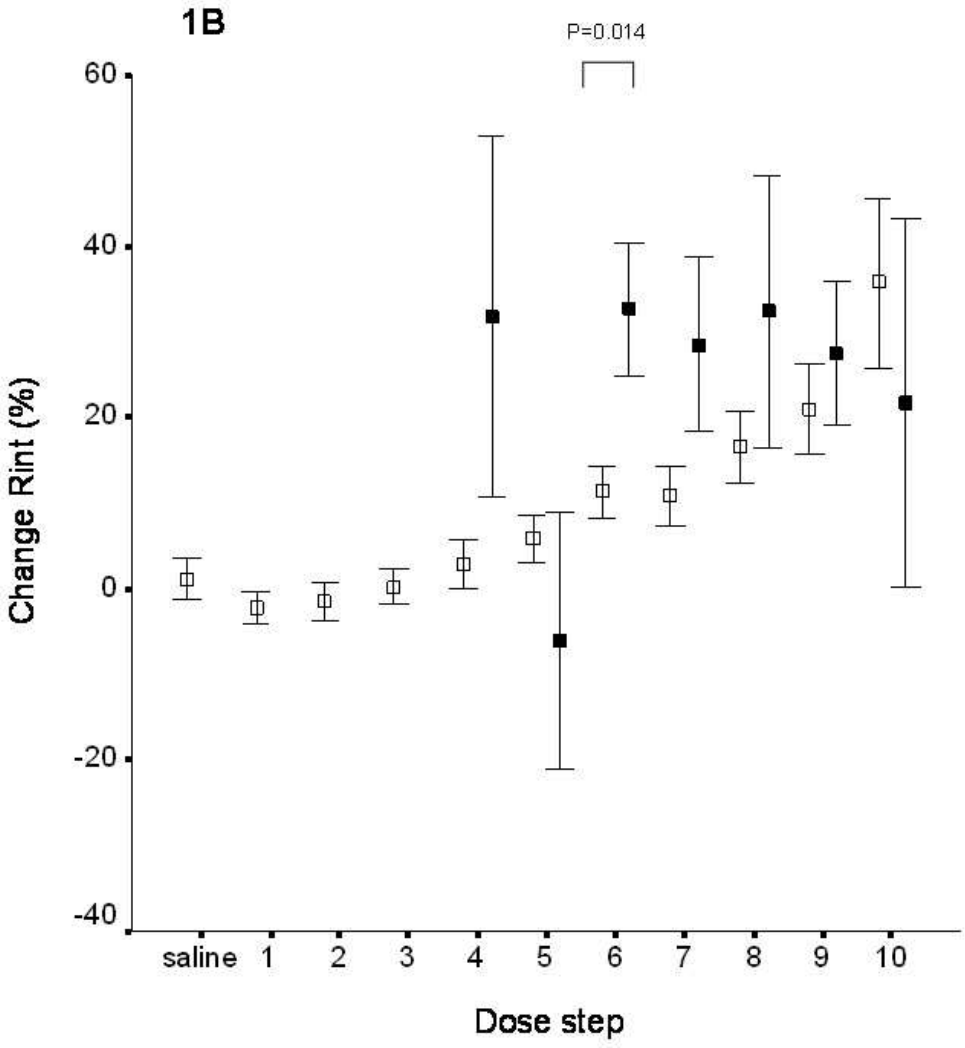


Figure 1. Mean changes from baseline of FEV_1 (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached at that dose step. Error bars represent SEM.
173x190mm (96 x 96 DPI)

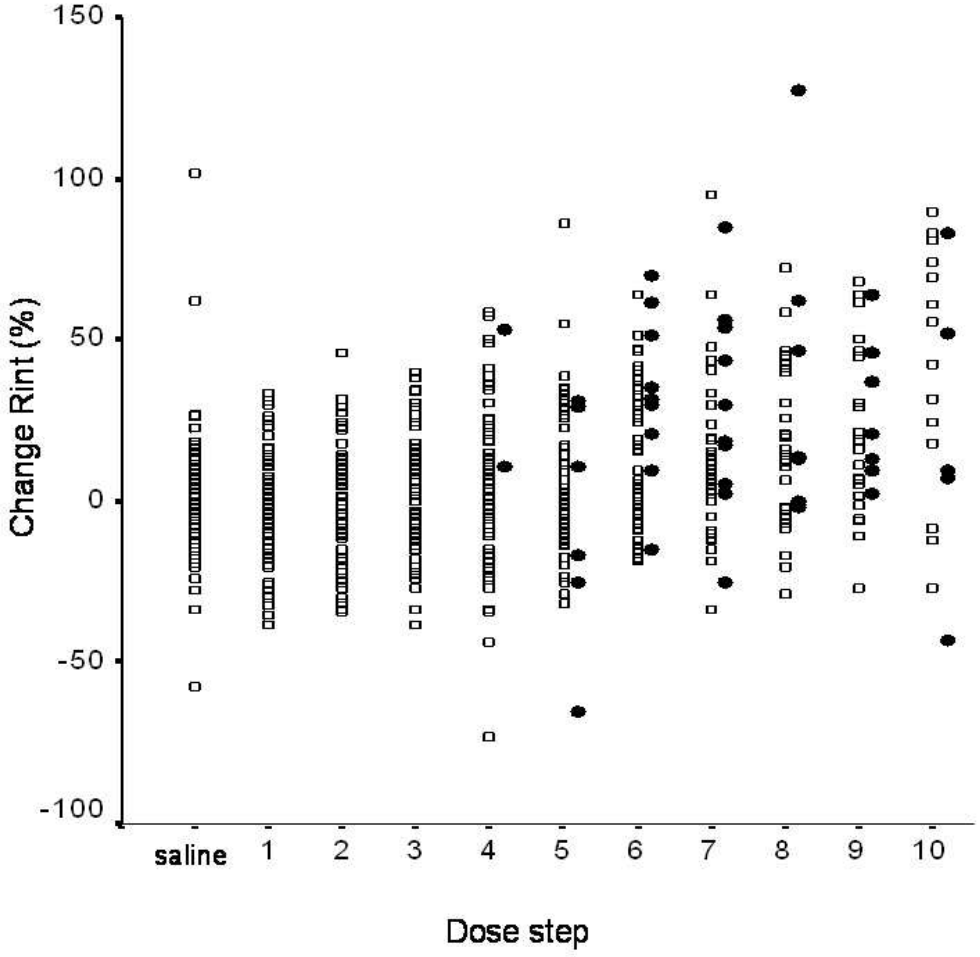


Figure 2. Individual changes from baseline of Rint at each dose step, divided according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached. At each dose step there is large overlap in changes from baseline of R_{int} between both groups.
191x190mm (96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

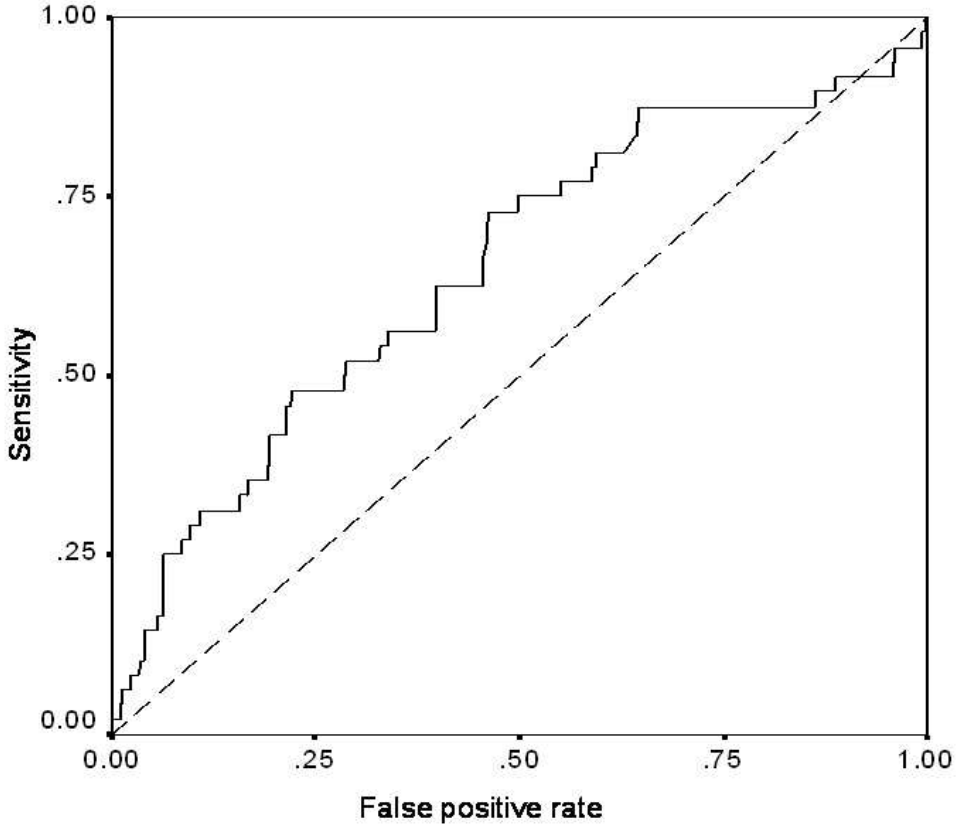


Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in FEV_1 (PD_{20}). Area under the curve (AUC) = 0.65.
186x161mm (96 x 96 DPI)

ONLINE DATA SUPPLEMENT

EVALUATION OF INTERRUPTER RESISTANCE IN METHACHOLINE CHALLENGE TESTING IN CHILDREN

Authors: Marije Koopman, MD[1]; Hein J.L. Brackel, MD, PhD[2]; Anja A.P.H. Vaessen-Verberne, MD, PhD[3]; Wim C. Hop, PhD[4]; Cornelis K. van der Ent, MD, PhD[1]; on behalf of the COMBO-Rint research group.

Institutions:

1. Department of Paediatric Pulmonology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands.
2. Department of Paediatrics, Catharina Hospital, Eindhoven, the Netherlands.
3. Department of Paediatrics, Amphia Hospital, Breda, the Netherlands
4. Department of Biostatistics, Erasmus MC – University Medical Centre Rotterdam, Rotterdam, the Netherlands

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 FEV_1 (PD_{20}) ≤ 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 FEV_1 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=15ms (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,

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 FEV_1 was selected. Reference values of Stanojevic (9) were used.

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

To quantify the variability of FEV_1 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 FEV_1 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 FEV_1 (E-figure 1).

E-REFERENCES

E1. Vaessen-Verberne A.A.P.H., Berg van den N.J., Nierop van J.C., Brackel H.J.L., Gerrits G.P.J.M., Hop W.C.J., Duiverman E.J. Combination therapy salmeterol/fluticasone versus doubling dose of fluticasone in children with asthma. *Am.J.Respir.Crit Care Med.* Accepted for publication. 2010.

E2. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. *J Allergy Clin Immunol* 2007 November;120(5 Suppl):S94-138.

E3. Birnie D, thoe Schwartzenberg GW, Hop WC, van Essen-Zandvliet EE, Kerrebijn KF. Does the outcome of the tidal breathing and dosimeter methods of assessing bronchial responsiveness in children with asthma depend on age? *Thorax* 1990 March;45(3):199-202.

E4. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000 January;161(1):309-29.

E5. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E et al. An official American Thoracic

Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007 June 15;175(12):1304-45.

E6. Phagoo SB, Wilson NM, Silverman M. Evaluation of the interrupter technique for measuring change in airway resistance in 5-year-old asthmatic children. *Pediatr Pulmonol* 1995 December;20(6):387-95.

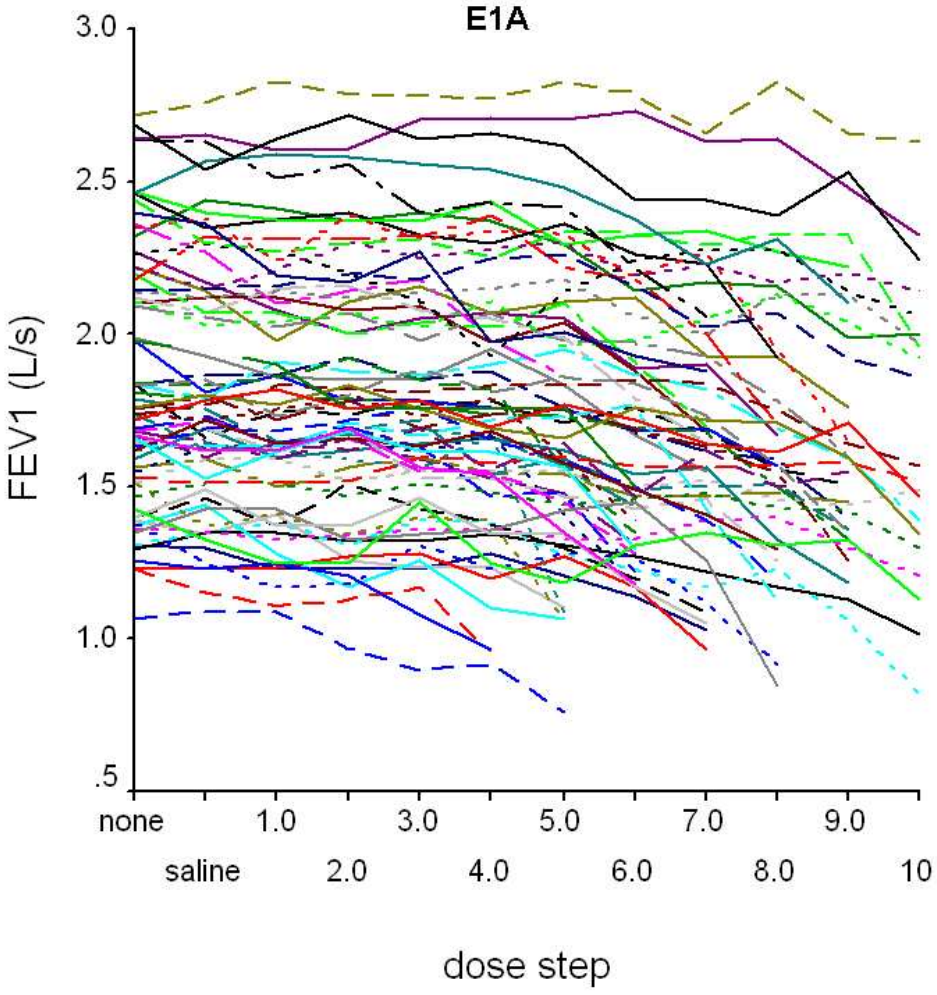
E7. Merkus PJ, Stocks J, Beydon N, Lombardi E, Jones M, McKenzie SA, Kivastik J, Arets BG, Stanojevic S. Reference ranges for interrupter resistance technique: the asthma UK initiative. *Eur Respir J* 2010 July;36(1):157-63.

E8. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J* 2005 August;26(2):319-38.

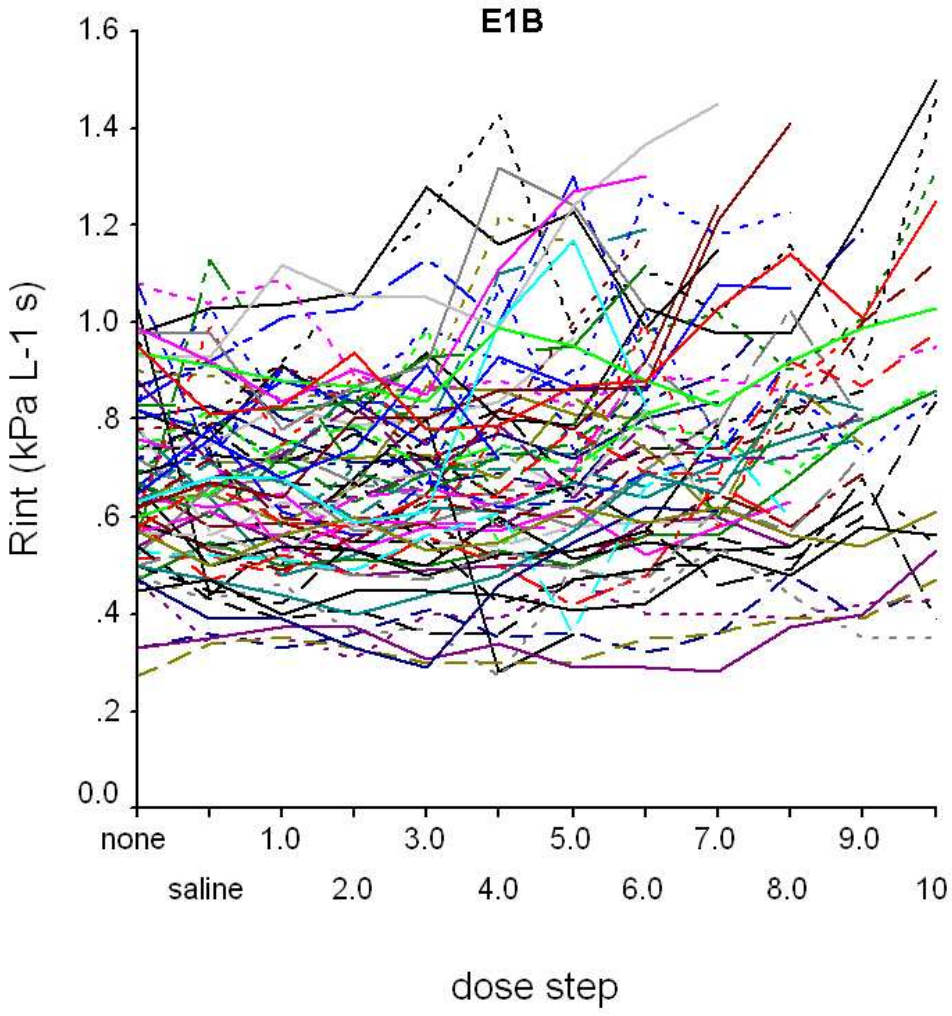
E9. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, Rosenthal M, Corey M, Lebecque P, Cole TJ. Reference ranges for spirometry across all ages: a new approach. *Am J Respir Crit Care Med* 2008 February 1;177(3):253-60.

E-FIGURE LEGEND

E-figure 1. Individual fluctuations of absolute values of FEV_1 (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 FEV_1 (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 FEV_1 (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)