Phenotype-driven Asthma Treatment in Children

Marianne Nuijsink

Promotiereeks HagaZiekenhuis

Het HagaZiekenhuis van Den Haag is trots op medewerkers die fundamentele bijdragen leveren aan de wetenschap en stimuleert hen daartoe. Om die reden biedt het HagaZiekenhuis promovendi de mogelijkheid hun dissertatie te publiceren in een speciale Haga uitgave, die onderdeel is van de promotiereeks van het HagaZiekenhuis. Daarnaast kunnen promovendi in het wetenschapsmagazine HagaScoop van het ziekenhuis aan het woord komen over hun promotieonderzoek.

Phenotype-driven Asthma Treatment in Children

2012 Den Haag

Vormgeving en opmaak
De VormCompagnie, Houten

Druk
DR&DV Media Services, Amsterdam

GlaxoSmithKline is gratefully acknowledged for their (financial) support of the work presented in this thesis.

Phenotype-driven Asthma Treatment in Children

Fenotypegestuurde astma behandeling bij kinderen

Proefschrift

ter verkrijging van de graad van doctor aan
de Erasmus Universiteit Rotterdam
op gezag van
de rector magnificus
Prof.dr. H.G. Schmidt
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
dinsdag 4 juni 2013 om 13.30 uur

door
Marianne Nuijsink

Promotiecommissie:

Promotoren:	Prof. dr. J.C. de Jongste
	Prof. dr. E.J. Duiverman
Overige leden:	Prof. dr. A.J. van der Heijden
	Prof. dr. C.K. van der Ent
	Prof. dr. R. Gerth van Wijk

Voor mijn ouders

Contents

Chapter 1

General introduction

1 General introduction

Asthma is a clinical syndrome characterized by recurrent episodes of wheezing, breathlessness, chest tightness and coughing, due to variable airflow obstruction. Chronic inflammation of the airway wall has been shown in asthma, mostly in adults, also between symptom episodes.[1] One of the pathophysiological consequences of airway inflammation in asthma is airway hyperresponsiveness (AHR).[2,3] Although inflammation is a consistent feature in asthma, at least in adults, the clinical spectrum of asthma is highly variable. Different symptom patterns have been demonstrated, various types of airway inflammation, and a large number of other clinical features that make asthma a heterogeneous disease.[1]

The goal of asthma treatment is good asthma control: control of clinical manifestations and of future risks such as exacerbations, rapid decline in lung function and side effects of medication.[4] Despite the availability of appropriate asthma medication and asthma management plans the burden of asthma symptoms in our population is still high and there is reason to try to develop better management strategies.[5-7] Better understanding of the pathophysiology of the various childhood asthma phenotypes is likely to improve asthma management and may enable us to individualize a patient's therapy. Lack of phenotype-based rather than disease-based approaches in the management of asthma may contribute substantially to the burden of asthma. Most studies of asthma treatment do not take phenotype into account. If a given treatment would be active for one phenotype but not for another, not taking separate phenotypes into account will lead to underestimation of treatment benefits. It is likely that one treatment does not fit all asthma patients.

Phenotypes of childhood asthma

Many attempts have been made to classify asthma in order to obtain homogeneous study populations. A wide range of features have been proposed for this purpose, such as severity of symptoms, exacerbations, allergic sensitization, AHR and response to bronchodilators.

Phenotypes and natural history of wheezing

In the Tuscon cohort, the time course of wheezing in the first years of life was used to define certain wheezing phenotypes.[8] Four distinct phenotypes were initially proposed on the basis of findings at 3 and 6 years of age: transient early wheezing, persistent wheezing, late-onset wheezing and no wheezing. Later studies, using more advanced statistical methods and a longer follow

up, confirmed and extended these original findings, and defined some more phenotypes.[9] Children with intermediate, late-onset and persistent wheezing phenotypes more often had an asthma diagnosis at later age, in contrast with children whose symptoms were transient in the first years of life. Unfortunately, knowledge about these temporal phenotypes has not been proven helpful for the clinician, because of the arbitrary definition and because it is not possible to identify these phenotypes prospectively. Furthermore, overlap in phenotypes and the fact that patients can move from one phenotype to another makes the clinical usefulness of this type of phenotyping questionable.[10,11]

Phenotypes and risk factors

An important aspect of defining phenotypes is that it allows for the identification of relevant risk factors. The first dichotomous phenotypes were allergic versus non-allergic asthma. However, allergic sensitization seems not to be an on-off phenomenon in the development of asthma.[12] Immunomodulating factors in early life and gene-environment interactions seem to be relevant for the development of childhood asthma.[13,14] Antenatal exposure to tobacco smoke is an example of a well documented risk factor for wheezing and asthma. It has its effect via impact on lung growth, immune maturation and perhaps epigenetic changes may be involved.[15]

The timing of exposure to e.g. allergens and viral infections is probably crucial for the development of asthma. But also the time point at which the outcome is established will determine the impact of a given risk factor. Synergistic interactions between viral lower respiratory infections and allergic sensitization in early life appear to be especially important in increasing the risk of subsequent asthma.[16] It is entirely unclear if asthma symptoms associated with early insults such as viral infection or passive smoking would benefit from other treatment modalities than symptoms due to other phenotypic characteristics, such as allergic sensitization.

Phenotypes and airway inflammation

About 30 years ago, eosinophilic airway inflammation was postulated as a central feature of asthma. In children, 50%-70% of asthma is associated with eosinophilic airway inflammation, depending on age.[17] Eosinophilic airway inflammation is associated with deterioration of asthma over time.[18] However, there has been increasing recognition that the inflammatory profile in asthma is heterogeneous. In stable childhood asthma two main phenotypes of airway inflammation were found: eosinophilic asthma (29-37%) and paucigranulocytic asthma,

where sputum eosinophils and neutrophils are within the normal range (46%-49%).[19-21] The clinical features of asthma may differ between these two phenotypes of airway inflammation.[20] Different from adult asthma, neutrophilic inflammation seems uncommon in stable childhood asthma.[22] Once the diagnosis of asthma is confirmed, the choice of treatment is commonly based on the severity of respiratory symptoms, without considering the presence and type of airway inflammation.[1,23]

Daily administration of inhaled corticosteroids (ICS) has become standard asthma therapy. ICS control symptoms, reduce airway inflammation, sputum eosinophils and exhaled nitric oxide (FeNO), decrease AHR and prevent airway remodeling that may lead to fixed airway obstruction. The changes in clinical markers and markers of airway inflammation have a different course in time that should be taken into account when evaluating treatment responses.[24] Most therapeutic trials do not take individual patient characteristics and treatment responses into account. We propose that asthma treatment studies should focus more on phenotype-driven asthma treatment. For example, patients with different phenotypes of airway inflammation may show differential responses to ICS.

Inflammatory phenotypes are difficult to establish in a clinical routine setting. Obviously, the most direct way to investigate the type of airway inflammation is by examining bronchial biopsies or broncho-alveolar lavage samples. However, this is not feasible in daily practice. Investigation of induced sputum samples has proven feasible and useful in adults, and is a second–best method to assess airway inflammation. However, in children the feasibility of getting appropriate sputum samples is low, especially in milder asthma, and the procedure of inducing sputum and working it up in the lab requires considerable expertise and is very time consuming.[25] The results of such tests take several days before they are available, and can therefore not be used for making therapy decisions on the spot. FeNO is an attractive clinical tool to assess airway inflammation, as it specifically reflects airway eosinophilia and can be measured quickly and accurately. However, studies using FeNO as a monitoring tool have until now been inconclusive.[26] It is unclear which noninvasive marker of airway inflammation would be most useful in routine patient care.

AHR can be used as a marker of airway inflammation. However, many mechanisms are potentially involved in AHR, such as airway inflammation and airway remodeling, including subendothelial fibrosis, smooth muscle hypertrophy, altered matrix composition and vascular changes.[27] Airway remodeling has

negative effect on airway mechanics, and may well contribute to AHR and is a risk factor for less favorable outcome of asthma.[28] This could make AHR a relevant target to treat in asthmatic patients, and a marker to monitor asthma treatment.

Phenotype-driven asthma treatment

In asthma treatment, the daily use of controller medication, especially ICS, aims to diminish airway inflammation. Adjustment of therapy is guided mainly by symptoms. However, there is a poor relation between symptoms, lung function and AHR.[29,30] Moreover, many patients with few symptoms do have AHR and signs of airway inflammation as evidence of an active disease process, and this may be harmful on the long run. It would make sense to titrate the dose of ICS on the presence of sputum eosinophilia. Indeed, a study in adult asthmatics using sputum eosinophils and symptoms to titrate ICS treatment showed a reduction of asthma exacerbations and admissions.[31] As sputum induction is not feasible in most children, this approach has not been shown effective in childhood asthma. It therefore remains to be shown if asthma treatment that specifically targets airway inflammation would prove superior to conventional, symptom-driven treatment in children.

Sont et al have postulated that titration of steroid treatment using repeated measurements of AHR might improve asthma therapy.[32] They showed in a prospective randomized controlled trial in adult asthmatics that a treatment strategy where repeated assessment of AHR was done, and steroid treatment adapted to the level of AHR would lead to better asthma control: a lower rate of asthma exacerbations, significantly greater improvement of forced expiratory volume in one second (FEV_1) and greater reduction in thickness of the subepithelial reticular layer. A number of studies has since confirmed that asthma treatment driven by inflammatory markers makes sense, and leads to clinically relevant improvements with fewer exacerbations and, sometimes, reduced steroid needs in adults.[26,33]

Aims of the studies described in this thesis

From the above we can conclude that current treatment of childhood asthma leaves room for improvement, especially by taking phenotypic features of the patients into account when treatment decisions are made. The aims of our studies were to establish any benefits of phenotype-driven asthma treatment in children, focusing on AHR as this is related to the presence of chronic airway inflammation and is clinically feasible. For that purpose we conducted a long-term therapeutic trial of AHR-driven asthma treatment of 2 years. We distinguished three asthmatic

phenotypes: children with symptoms and AHR, children with AHR with few symptoms and children who have symptoms but no AHR. By targeting steroid treatment to those with AHR, who might benefit most, we aimed to reduce over- and undertreatment, which is still common in children.[34] We investigated the effects of this treatment strategy on a variety of clinical and physiological outcomes, and assessed improvement of long-term evolution of the disease (chapter 2). In addition we documented the long-term effects of our 2-year intervention trial after an interval of 5 years (chapter 3). Good perception of bronchoconstriction is crucial in order to adequately report symptoms. Given the three defined phenotypes of our study population, based on symptoms and AHR, we explored the association between perception of airway obstruction, measured by Borg scores, and AHR (chapter 4).[35] As outlined before we need more non-invasive, feasible markers of inflammation in order to better phenotype asthmatic children, especially young children. Eosinophil protein X in urine (uEPX), a product of activated eosinophilic granulocytes, could serve as a non-invasive marker of airway inflammation. During our two-years intervention trial we collected a lot of data on clinical measures of asthma control, and measured urinary EPX. Chapter 5 reports the cross-sectional association between uEPX and clinical measures of asthma and airway inflammation. Chapter 6 describes uEPX changes in relation to changes of other markers of asthma and airway inflammation over time.

REFERENCES

1. Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2011. Available from: http://www.ginasthma.org/
2. Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Thorax. 1996;51:496-502
3. Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, Schwartz LB, Durham SR, Jeffery PK, Kay AB. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. J Allergy Clin Immunol. 1991;88:661-74
4. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, de Jongste JC, Kerstjens HA, Lazarus SC, Levy ML, O'Byrne PM, Partridge MR, Pavord ID, Sears MR, Sterk PJ, Stoloff SW, Sullivan SD, Szefler SJ, Thomas MD, Wenzel SE. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med. 2009;180:59-99

5. Gustafsson PM, Watson L, Davis KJ, Rabe KF. Poor asthma control in children: evidence from epidemiological surveys and implications for clinical practice. Int J Clin Pract. 2006;60:321-34
6. Rabe KF, Adachi M, Lai CK, Soriano JB, Vermeire PA, Weiss KB, Weiss ST. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. J Allergy Clin Immunol. 2004;114:40-7
7. Wildhaber J, Carroll WD, Brand PL. Global impact of asthma on children and adolescents' daily lives: the room to breathe survey. Pediatr Pulmonol. 2012;47:346-57
8. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med. 1995;332:133-8
9. Savenije OE, Granell R, Caudri D, Koppelman GH, Smit HA, Wijga A, de Jongste JC, Brunekreef B, Sterne JA, Postma DS, Henderson J, Kerkhof M. Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA. J Allergy Clin Immunol. 2011;127:1505-12 e14
10. Fleming L, Tsartsali L, Wilson N, Regamey N, Bush A. Sputum inflammatory phenotypes are not stable in children with asthma. Thorax. 2012;67:675-81
11. Schultz A, Devadason SG, Savenije OE, Sly PD, Le Souef PN, Brand PL. The transient value of classifying preschool wheeze into episodic viral wheeze and multiple trigger wheeze. Acta Paediatr. 2010;99:56-60
12. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, Gern JE, Gerritsen J, Hamelmann E, Helms PJ, Lemanske RF, Martinez F, Pedersen S, Renz H, Sampson H, von Mutius E, Wahn U, Holt PG. Early identification of atopy in the prediction of persistent asthma in children. Lancet. 2008;372:1100-6
13. Matricardi PM, Illi S, Gruber C, Keil T, Nickel R, Wahn U, Lau S. Wheezing in childhood: incidence, longitudinal patterns and factors predicting persistence. Eur Respir J. 2008;32:585-92
14. Sly PD, Holt PG. Role of innate immunity in the development of allergy and asthma. Curr Opin Allergy Clin Immunol. 2011;11:127-31
15. Anto JM, Pinart M, Akdis M, Auffray C, Bachert C, Basagana X, Carlsen KH, Guerra S, von Hertzen L, Illi S, Kauffmann F, Keil T, Kiley JP, Koppelman GH, Lupinek C, Martinez FD, Nawijn MC, Postma DS, Siroux V, Smit HA, Sterk PJ, Sunyer J, Valenta R, Valverde S, Akdis CA, Annesi-Maesano I, Ballester F, Benet M, Cambon-Thomsen A, Chatzi L, Coquet J, Demoly P, Gan W, Garcia-Aymerich J, Gimeno-Santos E, Guihenneuc-Jouyaux C, Haahtela T, Heinrich J, Herr M, Hohmann C, Jacquemin B, Just J, Kerkhof M, Kogevinas M, Kowalski ML, Lambrecht BN, Lau S, Lodrup Carlsen KC, Maier D, Momas I, Noel P, Oddie S, Palkonen S, Pin I, Porta D, Punturieri A, Ranciere F, Smith RA, Stanic B, Stein RT, van de Veen W, van Oosterhout AJ, Varraso R, Wickman M, Wijmenga C, Wright J, Yaman G, Zuberbier T, Bousquet J. Understanding the complexity of IgE-related phenotypes from childhood to young adulthood: a Mechanisms of the Development of Allergy (MeDALL) seminar. J Allergy Clin Immunol. 2012;129:943-54 e4
16. Holt PG, Strickland DH, Sly PD. Virus infection and allergy in the development of asthma: what is the connection? Curr Opin Allergy Clin Immunol. 2012;12:151-7
17. Gerritsen J. Follow-up studies of asthma from childhood to adulthood. Paediatr Respir Rev. 2002;3:184-92
18. Lovett CJ, Whitehead BF, Gibson PG. Eosinophilic airway inflammation and the prognosis of childhood asthma. Clin Exp Allergy. 2007;37:1594-601

19. Wang F, He XY, Baines KJ, Gunawardhana LP, Simpson JL, Li F, Gibson PG. Different inflammatory phenotypes in adults and children with acute asthma. Eur Respir J. 2011;38:567-74
20. Gibson PG, Simpson JL, Hankin R, Powell H, Henry RL. Relationship between induced sputum eosinophils and the clinical pattern of childhood asthma. Thorax. 2003;58:116-21
21. Gibson PG, Henry RL, Thomas P. Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate. Eur Respir J. 2000;16:1008-15
22. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology. 2006;11:54-61
23. British Thoracic Society/Scottish Intercollegiate Guidelines Network. British Guideline on the Management of Asthma. A National Clinical Guideline. 2012 www.sign.ac.uk/pdf/sign101.pdf
24. van Rensen EL, Straathof KC, Veselic-Charvat MA, Zwinderman AH, Bel EH, Sterk PJ. Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. Thorax. 1999;54:403-8
25. Garcia-Marcos L, Brand PL. The utility of sputum eosinophils and exhaled nitric oxide for monitoring asthma control with special attention to childhood asthma. Allergol Immunopathol (Madr). 2010;38:41-6
26. Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, Chang AB. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). Thorax. 2012;67:199-208
27. Sterk PJ, Bel EH. Bronchial hyperresponsiveness: the need for a distinction between hypersensitivity and excessive airway narrowing. Eur Respir J. 1989;2:267-74
28. Grol MH, Postma DS, Vonk JM, Schouten JP, Rijcken B, Koeter GH, Gerritsen J. Risk factors from childhood to adulthood for bronchial responsiveness at age 32-42 yr. Am J Respir Crit Care Med. 1999;160:150-6
29. Brusasco V, Crimi E, Pellegrino R. Airway hyperresponsiveness in asthma: not just a matter of airway inflammation. Thorax. 1998;53:992-8
30. Rosi E, Scano G. Association of sputum parameters with clinical and functional measurements in asthma. Thorax. 2000;55:235-8
31. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet. 2002;360:1715-21
32. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med. 1999;159:1043-51
33. Fleming L, Wilson N, Regamey N, Bush A. Use of sputum eosinophil counts to guide management in children with severe asthma. Thorax. 2012;67:193-8
34. Caudri D, Wijga AH, Smit HA, Koppelman GH, Kerkhof M, Hoekstra MO, Brunekreef B, de Jongste JC. Asthma symptoms and medication in the PIAMA birth cohort: evidence for under and overtreatment. Pediatr Allergy Immunol. 2011;22:652-9
35. Wilson RC, Jones PW. A comparison of the visual analogue scale and modified Borg scale for the measurement of dyspnoea during exercise. Clin Sci (Lond). 1989;76:277-82

Chapter 2

2

Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial

Marianne Nuijsink, Wim C. J. Hop, Peter J. Sterk, Eric J. Duiverman, Johan C. de Jongste on behalf of the CATO Study Group

Eur Respir J 2007; 30: 457-466

2 Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial

ABSTRACT

Background

Management plans for childhood asthma have limited success in optimizing asthma control. The aim of the present study was to assess, whether a treatment strategy guided by airway hyperresponsiveness (AHR) increased the number of symptom-free days and improved lung function in asthmatic children, compared with a symptom-driven reference strategy.

Methods

In a multicentre, double-blind, parallel group randomised 2-yr intervention trial, 210 children (aged 6-16y) with moderate atopic asthma, selected on the basis of symptom scores and/or presence of AHR were studied. At 3-monthly visits symptom scores, forced expiratory volume in one second (FEV_1) and methacholine challenge results were obtained, and medication (five levels of fluticasone with or without salmeterol) adjusted according to algorithms based on symptom score (reference strategy, n=104), or AHR and symptom score (AHR strategy, n=102).

Results

After 2 years, no difference was found in the percentage of symptom-free days between the treatment strategies. Pre-bronchodilator FEV_1 was higher in the AHR strategy (2·3% predicted, p=0·046). This was entirely explained by a gradual worsening of FEV_1 in a subgroup of 91 hyperresponsive children enrolled with low symptom scores (final difference between study arms was 6%).

Conclusion

Asthma treatment guided by airway hyperresponsiveness showed no benefits in terms of number of symptom-free days, but produced a better outcome of pre-bronchodilator forced expiratory volume in one second in allergic asthmatic children, especially those characterized by low symptom scores despite airway hyperresponsiveness.

INTRODUCTION

The long-term outcome of asthma depends upon the severity of the disease in early life; persistence into adulthood is more likely in children with more severe airway obstruction and airway hyperresponsiveness (AHR).[1] It has recently been shown that childhood asthma is associated with reduced lung function in young adults.[2] Therefore, treatment strategies that preserve lung function in addition to controlling symptoms in childhood may offer substantial long-term benefits. Despite effective therapies, asthma control appears far from optimal in a large proportion of asthmatics worldwide.[3] One of the explanations is inadequate suppression of airway inflammation. Inhaled corticosteroids (ICS) are the first choice anti-inflammatory treatment for asthma, suppress airway inflammation and improves symptoms and lung function.[4] According to Global Initiative for Asthma (GINA), ICS dosing is guided by symptoms and lung function.[5] However, the relation between these parameters and the severity of airway inflammation is weak or absent.[6] Moreover, several studies have shown persisting inflammation in the airways of well-controlled asthmatic patients despite treatment with ICS, and in patients in clinical remission of asthma.[7,8] These data indicate that not only symptoms or lung function but also inflammation should be considered when treating asthma with ICS. The validity of this concept is supported by a number of studies using different approaches.

In adult asthmatics, a treatment strategy focussing on suppression of sputum eosinophilia resulted in a reduction of asthma exacerbations.[9,10] Smith et al. found that titrating asthma therapy against exhaled nitric oxide fraction (FeNO), a marker of eosinophilic airway inflammation, resulted in a substantial reduction of ICS dose without compromising asthma control in adults.[11] In a recent paediatric study, Pijnenburg et al. showed that a significant improvement of airway hyperresponsiveness (AHR) could be reached by titrating ICS against FeNO, and this effect was obtained without increasing the mean ICS dose.[12] AHR to inhaled methacholine is associated with airway inflammation and remodelling.[7,13] Sont et al. showed that ICS dose titration guided by AHR reduced the asthma exacerbation rate by 1.8 fold, improved lung function and reduced remodelling in airway biopsy specimens from adult asthmatics.[14] AHR is a risk factor for rapid decline in lung function and severe AHR is associated with inadequate symptom perception.[1,15,16] Therefore it could be reasoned that asthmatic children with AHR and inadequate symptom perception are especially at risk for a rapid decline in lung function and might, therefore, particularly benefit from titrating asthma therapy against AHR. The present study tested the hypothesis that a treatment

strategy guided by AHR increases the number of asthma symptom-free days and provides better maintenance of lung function in children with moderately severe atopic asthma compared with a symptom-driven strategy.

METHODS

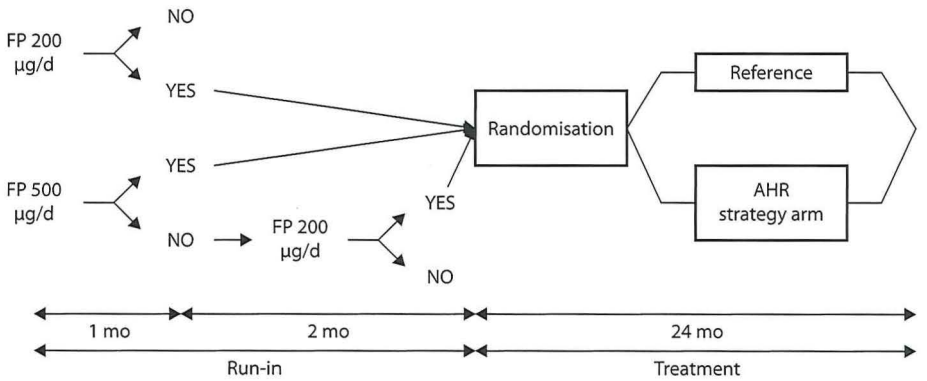
Patients

Clinically stable asthmatic children, living in the Netherlands, aged 6 -16 years with a documented clinical history of moderate persistent asthma according to GINA guidelines, were eligible and were enrolled from the 15 participating clinics, including 7 university medical centres. All patients gave a positive, class ≥ 1 radioallergosorbent test result for one or more airborne allergens and used ≥200 µg fluticasone/day or an equivalent dose of other ICS. The protocol allowed for inclusion of three different asthma phenotypes at randomisation1) Subgroup AHR (n-91 (44%)); children with a provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (FEV_1; (PD_{20}) < 150 µg and a cumulative symptom score (see below) of <14.2) Subgroup symptom (S; n=46, (22%)) children with a symptom score ≥ 14 and a PD_{20} ≥ 150 µg. Subgroup S+AHR (n=69, (34%)) children with a symptom score >14 and a PD_{20} < 150 µg. By including these different phenotypes, the results are applicable to the disease spectrum in paediatric clinical practice. All parents and children aged >12 yrs, signed their informed consent. The study was approved by the medical ethical committees of all participating centres.

Study design

The study was performed as a multi-centre, parallel group, randomised 2-yr intervention trial. Children were randomised after a run-in period of 1 month. In children treated with 500 µg fluticasone/day, who did not meet the criteria for randomisation after 1 month, the dose of ICS was tapered down to 200 µg fluticasone/day for another 2 months before randomisation. After run-in, children were randomised into one of 2 treatment strategy arms if they showed a cumulative symptom score ≥14 during the last 2 weeks of the run-in period and/or a PD_{20} of < 150 µg (figure 1).

Figure 1 *- Study design. The criteria for randomisation were a provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (PD_{20}) < 150 µg at the end of run-in and/or a cumulative symptom score ≥ 14 during the last 14 days of run-in. In the reference strategy arm, adjustment of treatment was based on the cumulative symptom score alone. In the airway hyperresponsiveness (AHR) strategy arm, treatment was adjusted according to PD_{20} and symptom score. FP: fluticasone dipropionate. 'Yes' and 'no' refer to either meeting the criteria for randomisation or not.*



The subjects were allocated in consecutive order. Randomisation was performed centrally using a minimisation program. Both treatment strategy groups were stratified for centre, baseline PD_{20} at the end of the run-in period (PD_{20} ≥ 150 µg or < 150 µg), cumulative symptom score at the end of the run-in (symptom score ≥14 or < 14), starting dose of fluticasone (100 bid or 250 bid) and age (<9 or ≥ 9 years).

In the reference strategy arm, adjustment of treatment was based on the cumulative symptom score alone. In the AHR strategy arm, treatment was adjusted on the basis of PD_{20} and symptom score, according to the algorithm shown in table 1, with PD_{20} cut-off levels 100 and 300 µg, corresponding to more severe and mild hyperresponsiveness, arbitrarily defined on the basis of current practise in the participating centres.

Table 1 *- Study algorithm. AHR: airway hyperresponsiveness; SS: cumulative symptom score during 14 days before the clinic visit. PD_{20}: provocative dose of methacholine causing 20% fall in forced expiratory volume in one second.*

Medication level	Reference strategy	AHR strategy
Increase by 1	SS ≥ 14	PD_{20} < 100 µg and SS < 14
		OR
		PD_{20} < 300 µg and SS ≥ 14
No change	0 < SS >14	PD_{20} 100 – 300 µg and SS < 14
		OR
		PD_{20} ≥ 300 µg and SS ≥ 14
Decrease by 1	SS = 0	PD_{20} > 300 µg and SS < 14

During the run-in phase, patients were put on 100 or 250 µg fluticasone bid depending on their equivalent treatment before run-in. After randomisation children were put on treatment according to level 3 or 4 of the algorithm. Every 3 months, the cumulative symptom score, lung function (FEV_1 and forced vital capacity (FVC)) and AHR were assessed by a lung function technician. Data were transmitted to a central computing facility which immediately produced a dosing advice according to the study algorithm. This advise was communicated to the physician who was blinded to outcomes and study arm allocation.

During the treatment period, subjects received the fluticasone dry powder inhaler (Flixotide Diskus®, GlaxoSmithKline, Brentford, UK) 100 or 200 µg per day (levels 1 and 2 respectively) or fluticasone/salmeterol multidose dry powder inhaler (Seretide Diskus®, GlaxoSmithKline) 200/100, 500/100 or 1000/100 µg per day (level 3, 4 or 5 respectively) (table 2).

Table 2 *- Study medication levels*

Level 1	fluticasone 100 µg od
Level 2	fluticasone 100 µg bid
Level 3	fluticasone 100 µg / salmeterol 50 µg bid
Level 4	fluticasone 250 µg / salmeterol 50 µg bid
Level 5	fluticasone 500 µg / salmeterol 50 µg bid

Medication levels were not blinded. For rapid relief of symptoms 200 µg salbutamol (Ventolin Diskus®, GlaxoSmithKline, UK) could be used. Subjects were asked to return all study medication at every visit, and remaining doses were counted. Compliance was defined as actually used doses divided by prescribed doses.

Symptom scores

During the study patients filled in a diary card daily for 2 weeks before each visit and for 3 months before the last visit.[17] Cough, shortness of breath and wheezing during night and day were recorded on a 4-point scale (0-3: no symptoms -severe symptoms interfering with activity or sleep). The maximum 14-days cumulative score was 252. For each diary card period the percentage of symptom-free days (defined as score 0 for cough, wheeze and shortness of breath) was determined.

Lung Function and Airway Hyperresponsiveness

Study medication was stopped 36 h before lung function and AHR testing. During each visit FEV_1 and FVC were measured.[18] AHR was tested by methacholine challenge using a dosimeter method.[19] Nebulised methacholine bromide was given in doubling oral doses of 1.52 – 1570 µg dose via a DeVilbiss 646 nebulizer (DeVilbiss Health Care Inc, Somerset, PA, USA) attached to a Rosenthal-French or a KOKO dosimeter (Laboratory for Applied Immunology, Fairfax, VA, USA). The PD_{20} was calculated from a log dose-response plot by linear interpolation of the data points. At the end of the challenge test, 400 µg Salbutamol was administered after a 20-minute pause and FEV_1 was measured again.

Asthma exacerbations

An exacerbation was defined as a deterioration of asthma requiring treatment with oral corticoids, as judged by the physician.[20]

Safety measures

Standing height was measured every 3 months using a wall-mounted Holtain stadiometer (Holtain Crymych, UK). If a child showed a decrease in height of 0.125 x SD (standard deviation score) in 6 months on two subsequent visits, the data were reviewed by an a independent paediatric endocrinologist (W.J.M. Gerver, Dept Paediatrics, Maastricht University Hospital, Maastricht, the Netherlands), and further evaluation could take place, including Tanner staging of puberty and radiological assessment of bone age. If serious growth problems were suspected, the patient could be withdrawn from the study.

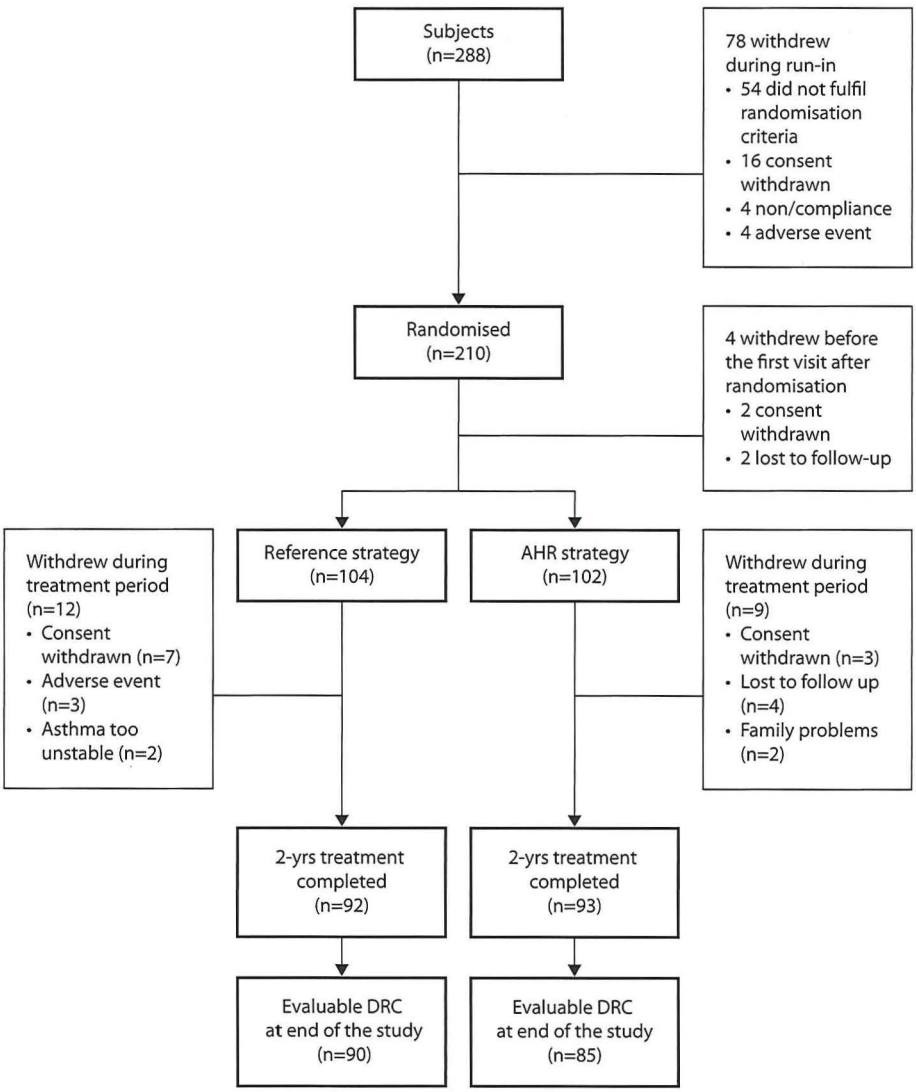
Statistical analysis

Diary card data were aggregated per patient for each of the 9 periods in the study (run-in, 7 subsequent periods of 14 days each prior to the clinic visit, followed by a final period of 3 months at the end of the study). Changes in the mean percentage of symptom-free days were compared between treatment strategies using repeated measures analysis of variance ANOVA (rMANOVA; with unstructured covariance matrix) with adjustment for baseline percentage during run-in. In the various rMANOVA models, time of assessment was included as a discrete factor. The mean percentage symptom free days during the final 3 months was the primary end-point. Power calculation was based on data from a previous study.[17] With two-sided α of 0.05, 100 patients in each group were needed to detect a 15% difference in symptom-free days at the end of the study with a power of 80%. Lung function (FEV_1(%predicted) and FEV_1/FVC x 100(%)) was also analyzed with rMANOVA, and the value measured at randomisation was used as a covariate. PD_{20} outcomes at the various visits were compared between study arms after logarithmic transformation. If, at a given visit, a patient did not reach a PD_{20} at the highest methacholine dose, this dose was considered as a right-censored observation in the analysis. The risk of exacerbation was compared between treatment strategies using the log-rank test.
Cumulative doses of ICS were compared between treatment strategies using the Mann-Whitney U-test. At baseline, a possibly relevant difference between the treatment strategies was found in the proportion of patients who had experienced exacerbations requiring prednisone treatment in the year before the study. Thus all analyses were done with an additional adjustment for this imbalance. Pre-planned subgroup analyses were performed for the three subgroups (AHR, S and S+AHR). Adjusted differences of means between treatment strategies cited in the present study always refer to the outcome in the AHR strategy arm minus the outcome in the reference strategy arm.
All analyses were done according to the intention-to-treat principle. A p-value of 0·05 (two-sided) was considered the limit of significance.

RESULTS

Between December 1999 and November 2003, 288 children entered the run-in, of whom 210 fulfilled the criteria for randomisation. As four patients withdrew within the first 3 months after randomisation and no follow up data were available, 206 could be evaluated (figure 2).

Figure 2 *- Trial profile. AHR: airway hyerresponsiveness; DRC: daily record card.*



The characteristics of the children in both treatment strategy arms and in the three subgroups are summarized in table 3.

Table 3 *- Anthropometric data of subjects in two treatment strategies and three subgroups. Data are presented as mean ± SD or median (range), unless otherwise stated. AHR; airway hyperresponsiveness; S: symptom; ICS inhaled corticosteroid; FEV_1: forced expiratory volume in one second; % predicted; FVC: forced vital capacity; PEF: peak expiratory flow; PD_{20}: provocative dose of methacholine causing a 20% fall in FEV_1; SS: cumulative symptom score during the last 14 days before the clinic visit. *: well controlled symptoms and AHR during run-in. ** manifest symptoms without AHR during run-in; *** manifest symptoms and AHR during run-in.*

	Strategy		Subgroups		
	Reference	**AHR**	**AHR***	**S****	**S+AHR*****
Subjects n	104	102	91	46	69
Males/females n	54/50	63/39	54/37	30/16	33/36
Age yrs	10·9 (2·5)	10·8 (2·4)	10·7 (2·5)	11·0 (2·1)	11·0 (2·5)
Duration of asthma yrs	7·1 (3·0)	7·3 (3·1)	7·3 (3·0)	7·0 (3·3)	7.3 (3·0)
Duration of ICS usage yrs	5·2 (2·7)	5·8 (2·7)	5·4 (2·9)	5·4 (3·0)	5·7 (2·4)
Asthma exacerbations in year prior to the study n	12	25	20	5	12
FEV_1 %pred	98 (14)	96 (14)	97 (15)	100 (12)	94 (14)
FEV_1/FVC %	83 (8)	81(9)	81 (9)	86 (6)	81 (8)
PEF-variability %	7 (4)	7 (4)	6 (3)	7 (5)	8 (5)
PD_{20} µg	73	68	47	553	42
	[0·8->1570]	[0·8->1570]	[3-148]	[154->1570]	[0·8-144]
Run-in SS	17	17	2	28	31
	[0-87]	[0-152]	[0-13]	[14-100]	[15-152]

At baseline the patients in both arms were similar, except for a higher number of children in the AHR strategy arm who had experienced one or more asthma exacerbations in the year prior to the study.

Symptom-free days

The number of patients with an evaluable diary decreased from baseline to end of the study from 104 to 90 for the reference strategy, and from 102 to 85 for the AHR strategy. Within each treatment strategy arm, the percentage symptom-free days increased similarly. The mean ± SEM percentage of symptom-free days within the reference and AHR strategy arms rose from 50±4 to 71± 3 and from 47±3 to 69±3 respectively (both p<0·001; figure 3A). The adjusted difference in the mean percentage symptom-free days between the strategy arms during the last 3 months was -1·1 (95% conference interval (CI) -10·1 to 7·9%, p=0·84). Within the three subgroups this difference was also nonsignificant (+3·1% (p=0·63), -11·9% (p=0·26) and -3·2% (p=0.72)) for subgroups AHR, S and S+AHR, respectively ; figure 3B-D)).

Figure 3 *- Symptom-free days as a function of time during follow-up (■: reference strategy; •: airway hyperresponsiveness (AHR) strategy)in A) total study population (n=206);B) AHR Subgroup (AHR and well-controlled symptoms during run-in; n=91) C); symptom (S) subgroup (manifest symptoms without AHR during run in; (n=46); and D). S+AHR subgroup (manifest symptoms and AHR during run in; n=69). Error bars indicate the SEM at each visit. The adjusted difference in the mean percentage of symptom-free days during the last 3 months did not differ between the strategy arms.*

Figure 3A

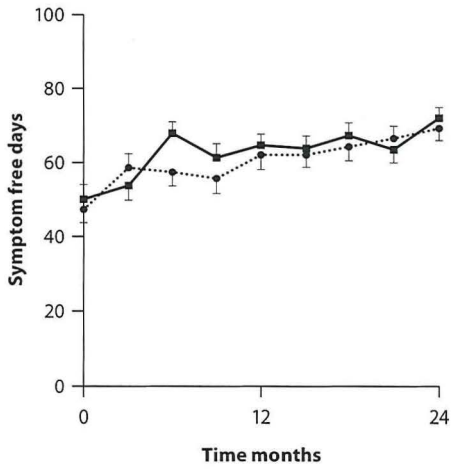


Figure 3B

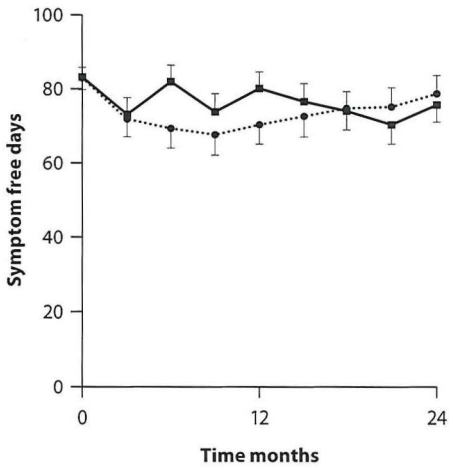


Figure 3C

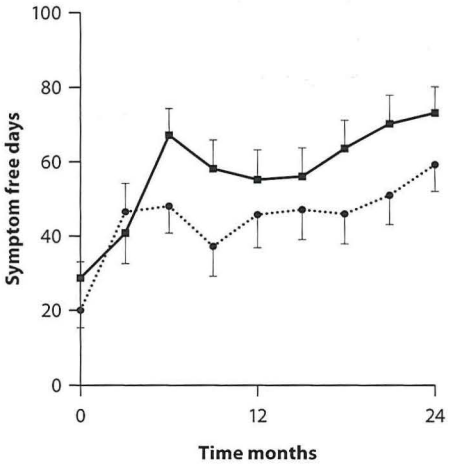
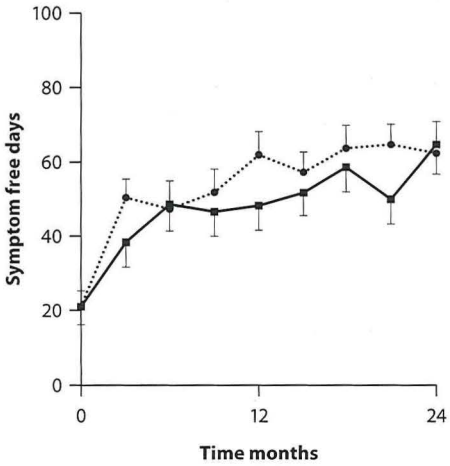


Figure 3D



Lung function and airway hyperresponsiveness

During the study the number of patients with valid lung function results decreased from 101 to 92 in the reference strategy and from 99 to 93 in the AHR strategy. Overall, the adjusted mean FEV_1(95% CI) at the eight visits after randomisation was 2·3 (0.05-4.6) percentage points higher in the AHR strategy arm (p=0·046; figure 4A). Children with AHR and well-controlled symptoms at randomisation (subgroup AHR) had a significantly higher FEV_1 in the AHR strategy arm than in the reference strategy arm (p=0·024). The adjusted difference in FEV_1 between the two treatment strategies in the AHR subgroup after 2 yrs of treatment was 6·0(1·2 - 10·8)% pred in favour of the AHR strategy arm (p=0·017; fig 4B). This difference was explained by a gradual decrease in the reference strategy arm from 97±2% pred at randomisation to 93±2% after 2 yrs (p=0·027), whereas FEV_1 did not change within the AHR strategy arm, (97± 2 to 98± 2% pred; p=0·23).

Figure 4 *- Change (Δ) from baseline in forced expiratory volume in one second (FEV_1) as a function of time during follow-up (■: reference strategy; •:airway hyperresponsiveness (AHR) strategy) in: A) total study population (n=206); and B) AHR subgroup (AHR and well-controlled symptoms during run in; n=91). Error bars represent SEM. During the treatment period, the mean difference in change from baseline FEV_1 was 2·3%pred in the total study population (p=0·046).*

Figure 4A

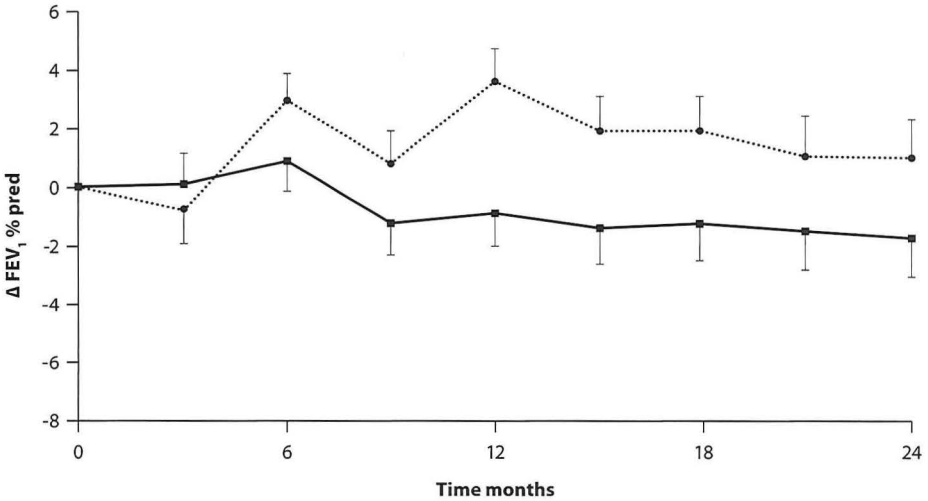
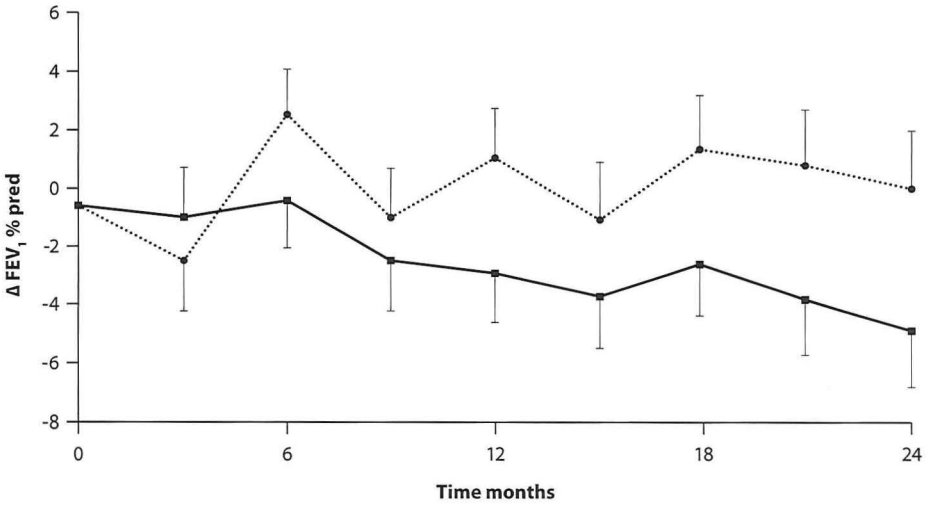


Figure 4B



After 2 yrs of treatment, the adjusted difference in change from baseline FEV_1 between the two treatment strategies was 6·0 %pred in the AHR subgroup (p=0·017)

In subgroup S and S+AHR there were no significant changes in FEV_1 between treatment strategies after 2 years (-2·0% (p=0·55) and -0·15% (p=0·98) respectively). Of the two results the first was borderline significantly different (p=0·052) from the corresponding difference of 6·0% in the AHR subgroup. In subgroup AHR the mean FEV_1/FVC at the end of the study was 1·6(0·3 to 2·9) percentage points higher in the AHR strategy arm (p=0·015). In the other two subgroups (S and S+AHR), differences were not significant (both p>0·17). The PD_{20} increased significantly during the treatment period. No difference was found between reference and AHR strategies (the changes from baseline at 2 yrs was 2·6 and 2·8 doubling doses respectively).

Treatment strategies and ICS dose

In the AHR strategy arm, 47% of the decisions would have been different had patients been treated according to the reference strategy: it would have led to a higher medication level in 24% and to a lower level in 23%. The mean daily ICS dose was 478± 27 µg fluticasone in the reference strategy arm and 562± 26 µg in the AHR arm (p= 0·025). However, at the end of the study, the difference between the treatment strategy arms was nonsignificant (505 µg/day in the reference strategy, and 557 µg/day in the AHR strategy;, p= 0·428; Figure 5A) The largest difference between strategies was seen after 6 months in subgroup AHR (figure 5B).

Figure 5 *- Daily fluticasone propionate (FP) dose during follow-up. (■: reference strategy; •: airway hyperresponsiveness (AHR) strategy) in: a) total study population (n=206); b) AHR subgroup (AHR (provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (PD_{20}) < 150 µg) and well-controlled symptoms (cumulative symptom score <14) during run-in; n=91(44%)); c) symptom (S) subgroup (manifest symptoms during run in; n=46 (22%)); and d) S+AHR subgroup (manifest symptoms (symptom score ≥ 14) and AHR (PD_{20} < 150µg) during run-in; n=69). Error bars represent SEM.*

During the study, the mean daily FP dose in the study population differed between the strategy arms (p= 0·025); at the end of the study this difference was no longer significant (p= 0·428). During and at the end of the study, the mean daily FP in the AHR subgroup differed between the strategy arms (p< 0·001).

Figure 5A

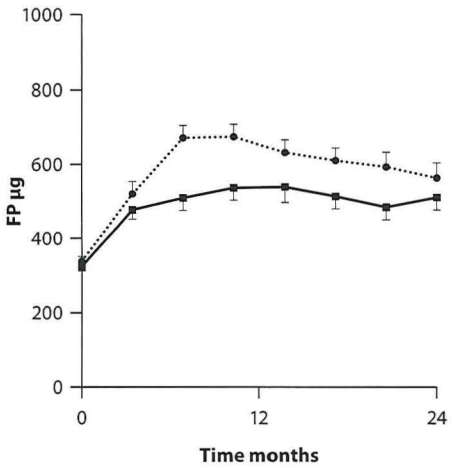


Figure 5B

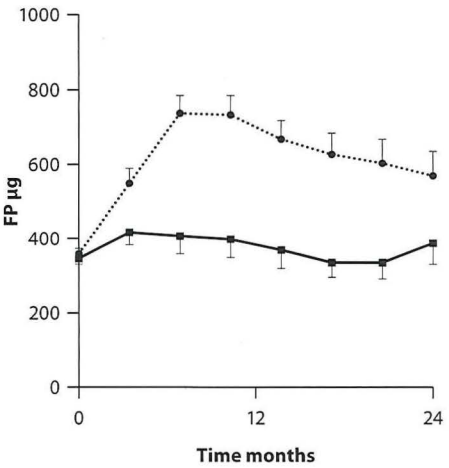


Figure 5C

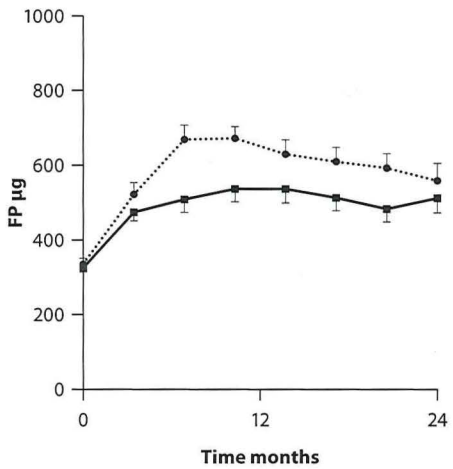
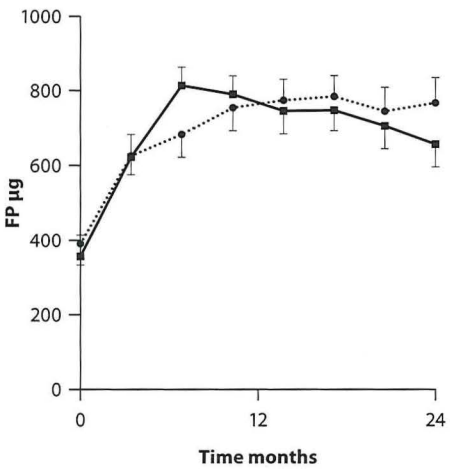


Figure 5D



The mean±SD compliance with treatment was excellent, 89±9% for the reference strategy arm and 88±8% in the AHR strategy arm. Compliance was equally good in children with and without symptoms (subgroup S and S+AHR versus subgroup AHR).

Asthma exacerbations

During the study, 17 out of 104 subjects in the reference strategy arm experienced at least one asthma exacerbation, versus 16 out of 102 in the AHR strategy arm (p=0·69). During the year before the study these figures were 12 and 25, respectively.

Side-effects

At the end of the study no significant difference in change of growth rate was found between the treatment strategies (baseline adjusted difference -0·1 x SDS, (95%CI: -0·2 to +0·04); p=0·18). However, in the subgroup AHR, a significant height difference was found between the treatment strategies at the end of the study (adjusted difference of -0·4 x SDS (95% CI -0·6 to -0·2); p<0·001). Growth data were no reason for discontinuing the study in any subject.

DISCUSSION

We have shown that, in allergic asthmatic children, 2 yrs of AHR-driven treatment did not improve the percentage of symptom-free days, but resulted in better pre-bronchodilator FEV_1, especially in a large subgroup whose asthma was characterized by AHR and low symptom scores. Adjusting maintenance treatment solely based on symptoms in this subgroup produced a gradual deterioration of pre-bronchodilator FEV_1 during the treatment period of 2 years.

Inflammation is associated with structural changes in the airways, and one of the major functional consequences of airway inflammation and remodeling is considered to be AHR.[13] Remodeling has been found in early stages of childhood asthma, and it is not known whether remodeling is responsible for long-term changes in lung function in childhood asthma.[21] However, prolonged ICS treatment results in progressive improvement of AHR.[22] This might be an important therapeutic objective, since AHR correlates with the long-term prognosis of lung function development and with the risk of persistence of disease into adulthood.[23,25] Indeed, young adult asthmatics already show loss of lung function in comparison with controls.[2] Late introduction of ICS or inadequate dosing may well promote such lung function impairment.[23]

Several studies have examined long-term effects of ICS in children with asthma. The Childhood Asthma Management Program (CAMP) study found that ICS improved pre- but not postbronchodilator FEV_1 compared with placebo, suggesting an effect of treatment on smooth muscle tone rather than airway structure, and no loss of lung function.[26] The prebronchodilator FEV_1/FVC ratio was significantly higher in steroid-treated children compared with the placebo group, consistent with less airway obstruction. Indeed, the large inhaled Steroid Treatment As Regular Therapy (START) in early asthma study, showed a small increase in pre-bronchodilator FEV_1 as a result of steroid treatment when comparing ICS and placebo.[27] Both studies were essentially different from the present one, in which the focus was not on ICS versus placebo but on treatment strategies. In addition, we studied children who were already being treated with ICS in specialist practices prior to the study, and used higher inhaled steroid doses than CAMP or START studies. It is remarkable that there was no reduction in FEV_1 with placebo treatment in both studies; indeed, a small increase was observed. The present authors speculate that this may be related to initial asthma severity. Merkus et al. examined the effect of ICS on the progression of FEV_1 in children aged > 3 years, and showed preservation of lung function compared with

placebo.[28] In children receiving placebo, pre- and post-bronchodilator values ran largely in parallel, whereas a ceiling effect towards a sub maximal level was seen with ICS.

How can the beneficial effect on lung function of treatment that is guided by AHR be explained? Conflicting data on the effects of medium- and long-term treatment with ICS on airway remodelling have been published.[14,29,30] AHR is a risk factor for a rapid decline in lung function in asthma, and, moreover, the decline in lung function with age in asthmatics correlates with the severity of AHR.[1,22,31] Several studies have shown that AHR is inversely associated with growth of airway calibre; therefore, it is possible that airway growth benefits from the AHR strategy.[32] The present authors speculate that the gradual and consistent effects on FEV_1 of the AHR strategy represent a preventive and, perhaps, therapeutic effect of ICS on airway remodelling.

Using both treatment strategies, the percentage of symptom-free days increased. Possible reasons include an effect of medication, increased compliance with treatment or spontaneous improvement of disease. An effect of treatment seems plausible, and could be caused by ICS or long-acting β2 agonists (LABAs). Most of the time, LABAs were used by most children according to GINA guidelines and could have reduced symptoms. At baseline, symptom scores were unaffected by LABAs as these were not allowed during run-in. The study design did not allow for disentangling the effects of ICS and LABAs.

AHR was defined on the basis of fixed cut-off levels, and it may be argued that this is inappropriate for children of a wide age range. LeSouëf reasoned that, merely as a result of different lung size, the same dose of methacholine would have a stronger effect in smaller children.[33] In the absence of age-specific data for determining AHR there is currently no means of solving this problem. The impact on the present study may be limited as the treatment arms were stratified for age. However, it is probable that selection on a fixed level of AHR has favoured the inclusion of young patients with less severe AHR.

Of the present study population, 44% initially had low symptom scores despite AHR, suggesting that this phenotype is common in the present secondary and tertiary care setting. Pre-planned analysis of this subgroup revealed that the AHR strategy, but not the reference strategy, prevented worsening of prebronchodilator FEV_1 over 2 years follow-up. In the remaining children asthma outcome could not be improved by taking AHR into account. The present

treatment algorithms predictably resulted in an increase in ICS dose in children with well-controlled symptoms treated according to the AHR strategy. A transient difference in ICS dose between the treatment strategies was seen over the course of the 2 yrs of treatment. This indicates that the AHR strategy was able to selectively improve the targeting of ICS to children in whom the reference strategy seems suboptimal. Overall, the increase in ICS dose was entirely explained by dose changes during the first 2 visits following randomisation. The cut-off symptom score at which the level of medication had to be increased was determined on the basis of a previous study using the same diary card.[17] The median symptom score at the start of the present study was higher than the predefined cut off level suggesting more severe disease in the present study than in our previous one. Studies published recently show that the dose-response relation of inhaled fluticasone with symptoms and FEV_1 reaches a plateau at 200-400 μg per day.[34] Based on these data, the present highest level of study medication could be questioned. However, not only dose, but also inhaler technique and means of delivery of ICS determines their efficacy, and data from adults may not apply to children. More recently, systemic side effects of fluticasone have been reported at doses ≥ 500 ug per day or higher.[35] At the onset of the present study fluticasone doses of ≤ 1000 ug per day were common practice in the participating clinics, and it was felt appropriate to continue this practise during the study. Whether or not similar long-term benefit could be derived from lower doses of ICS in children warrants further study.

In the present study, children were treated in line with GINA guidelines, often combining LABAs and an ICS. As LABAs improve symptoms but not inflammation, it could be argued that this has affected assignments to subgroups and symptom scores. However, baseline symptom scores were obtained during the run-in period when LABAs were not allowed. Furthermore, all lung function tests were performed ≥36 h after the last dose of study medication and therefore not influenced by LABAs. Finally, the cumulative proportion of children treated with LABAs was not different between treatment strategies, and it seems likely that any effect of LABA on symptoms will have been similar in both study arms. Consequently the present authors are confident that the use of LABAs has not systematically biased the results of the present comparisons.

No adverse effects of ICS treatment on statural growth was found in the group as a whole, but observed a slight-but-significant reduction in growth velocity in the AHR subgroup who were treated with relatively high ICS doses. Previous studies designed to evaluate growth in asthmatic children have shown that growth rates

are commonly reduced during the first months of ICS treatment, but that this does not affect adult height; thus, a negative effect on adult height is unlikely.[36] What is the clinical implication of the present study? Asthmatic children characterized by AHR and well-controlled symptoms are obviously at risk for suboptimal treatment when treated according to the severity of their symptoms. However, measurement of AHR is currently rare in pediatric practice and is time consuming. The present authors would not advocate routine AHR measurements in all asthmatic children for that reason. However, AHR testing may be worthwhile in asthmatic children who report few symptoms, in order to identify those at risk for lung function deterioration.

In conclusion, it was found that an airway hyperresponsiveness-driven asthma treatment, as compared with the conventional symptom-driven strategy, produced no improvement in the percentage of symptom-free days, but prevented long-term worsening of prebronchodilator forced expiratory volume in one second, specifically in a large subgroup of children who show airway hyperresponsiveness and low symptom scores. This effect on lung function was achieved with higher doses of inhaled corticosteroids. The present authors speculate that phenotype-specific treatment strategies have the potential to improve the outcome of childhood asthma into adult life.

REFERENCES

1. Grol MH, Gerritsen J, Vonk JM, et al. Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. A 30-year follow-up study. Am J Respir Crit Care Med 1999; 160: 1830-1837.
2. James AL, Palmer LJ, Kicic E, et al. Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. Am J Respir Crit Care Med 2005; 171: 109-114.
3. Rabe KF, Adachi M, Lai CK, et al. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. J Allergy Clin Immunol 2004; 114: 40-47.
4. Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids. New developments. Am J Respir Crit Care Med 1998; 157: S1-S53.
5. National Institutes of Health, National Heart, Lung, and Blood Institute. Global initiative for asthma. Global Strategy for Asthma Management and Prevention NIH Publication No 02-3659 Issued January, 1995: updated 2004.
6. Djukanovic R, Wilson JW, Britten KM, et al. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. Am Rev Respir Dis 1992; 145: 669-674.
7. Sont JK, Han J, van Krieken JM, et al. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Thorax 1996; 51: 496-502.

8. van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med 2001; 164: 2107-2113.
9. Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002; 360: 1715-1721.
10. Jayaram L, Pizzichini MM, Cook RJ, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. Eur Respir J; 2006: 27: 483-494.
11. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. N Engl J Med 2005; 352: 2163-2173.
12. Pijnenburg MW, Bakker EM, Hop WC, de Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomised controlled trial. Am J Respir Crit Care Med 2005; 172: 831-836.
13. Boulet LP, Chakir J, Dube J, Laprise C, Boutet M, Laviolette M. Airway inflammation and structural changes in airway hyper-responsiveness and asthma: an overview. Can Respir J 1998; 5: 16-21.
14. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med 1999; 159: 1043-1051.
15. Bijl-Hofland ID, Cloosterman SG, Folgering HT, Akkermans RP, van Schayck CP. Relation of the perception of airway obstruction to the severity of asthma. Thorax 1999; 54: 15-19.
16. Koh YI, Choi IS, Lim H. Airway responsiveness as a direct factor contributing to the dyspnoea perception in asthma. Respir Med 2001; 95: 464-470.
17. Verberne AA, Frost C, Roorda RJ, van der Laag H, Kerrebijn KF. One year treatment with salmeterol compared with beclomethasone in children with asthma. The Dutch Paediatric Asthma Study Group. Am J Respir Crit Care Med 1997; 156: 688-695.
18. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16: 5-40.
19. 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; 45: 199-202.
20. Pauwels RA, Lofdahl CG, Postma DS, et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. N Engl J Med 1997; 337: 1405-1411.
21. Payne DN, Rogers AV, Adelroth E, et al. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med 2003; 167: 78-82.
22. Woolcock AJ, Yan K, Salome CM. Effect of therapy on bronchial hyperresponsiveness in the long-term management of asthma. Clin Allergy 1988; 18: 165-176.
23. Sears MR, Greene JM, Willan AR, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003; 349: 1414-1422.
24. Strunk RC, Weiss ST, Yates KP, et al. Mild to moderate asthma affects lung growth in children and adolescents. J Allergy Clin Immunol 2006; 118: 1040-1047.

25. de Marco R, Marcon A, Jarvis D, et al.Inhaled steroids are associated with reduced lung function decline in subjects with asthma with elevated total IgE. J Allergy Clin Immunol. 2007; 119: 611-617.
26. Long-term effects of budesonide or nedocromil in children with asthma. The Childhood Asthma Management Program Research Group. N Engl J Med 2000; 343: 1054-1063.
27. Pauwels R A, Pedersen S, Busse WW, et al. Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial. Lancet 2003; 361: 1071-1076.
28. Merkus PJ, van Pelt W, van Houwelingen JC, et al. Inhaled corticosteroids and growth of airway function in asthmatic children. Eur Respir J 2004; 23: 861-868.
29. Ward C, Pais M, Bish R, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. Thorax 2002; 57: 309-316.
30. Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson SA. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma. A quantitative light and electron microscopic study. Am Rev Respir Dis 1992; 145: 890-899.
31. Rasmussen F, Taylor DR, Flannery EM, et al. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV_1/vital capacity ratio: a longitudinal population study from childhood to adulthood. Am J Respir Crit Care Med 2002; 165: 1480-1488.
32. Xuan W, Peat JK, Toelle BG, Marks GB, Berry G, Woolcock AJ. Lung function growth and its relation to airway hyperresponsiveness and recent wheeze. Results from a longitudinal population study. Am J Respir Crit Care Med 2000; 161: 1820-1824.
33. Le Souef PN. Can measurements of airway responsiveness be standardized in children? Eur Respir J 1993; 6: 1085-1087.
34. Masoli M, Weatherall M, Holt S, Beasley R. Systematic review of the dose-response relation of inhaled fluticasone propionate. Arch Dis Child 2004; 89: 902-207.
35. Levine LS, Boston BA. Effect of inhaled corticosteroids on the hypothalamic-pituitary-adrenal axis and growth in children. J Pediatr 2000; 137: 450-454.
36. Brand, P. L. Inhaled corticosteroids reduce growth. Or do they? Eur Respir J 2001; 17: 287-294.

Chapter 3

Long-term follow-up after 2 years of asthma treatment guided by airway responsiveness in children

Marianne Nuijsink, Anja A.P.H. Vaessen-Verberne,
Wim C. J. Hop, Peter J. Sterk, Eric J. Duiverman,
Johan C. de Jongste on behalf of the CATO Study Group

In press Respiratory Medicine

3 Long-term follow-up after 2 years of asthma treatment guided by airway responsiveness in children

ABSTRACT

Introduction

Children with persistent asthma may have diminished lung function in early adulthood. In our previous study ('CATO') we showed preservation of lung function in asthmatic children, during 2 years of treatment that was guided by airway hyperresponsiveness (AHR). The aim of the present prospective follow up study was to investigate whether the positive effect of the AHR strategy on lung function had persisted beyond the duration of the intervention study, after several years of usual care by paediatrician and general practitioner.

Methods

With a mean interval of 4.4 y after the last visit, 137 subjects (67% of the original CATO population) participated in this follow-up study. Evaluation consisted of spirometry (n=137), a methacholine challenge test (n=83), data on inhaled steroid treatment and asthma exacerbations (n=137), and an asthma symptom diary during 6 weeks (n=90).

Results

At follow-up, lung function, % symptom-free days and exacerbation rates of both treatment strategy groups was similar. The mean dose of inhaled corticosteroids had diminished from 550 µg/day at the end of CATO to 235 µg/day at follow-up. The decrease in AHR measured at the end of CATO was maintained at follow-up for both treatment strategy groups.

Conclusion

The beneficial effect on lung function of 2 years treatment guided by AHR was lost after 3-7 years of usual care. This suggests that an AHR-guided treatment strategy may need to be sustained in order to preserve lung function.

INTRODUCTION

Some longitudinal studies have shown that children with persistent asthma have a lower lung function in young adulthood.[1,2] This seems independent of therapy with inhaled corticosteroids (ICS).[3] The outcome of asthma depends on the duration of the disease[4,5], airflow obstruction[6,7], level of airway hyperresponsiveness (AHR)[8], and gender.[1] Treatment with ICS reduces airway inflammation and symptoms and improves lung function.[9,10] Treatment strategies that titrated the dose of ICS on the presence and severity of eosinophilic airway inflammation showed marked reduction of asthma exacerbations[11,12] and AHR.[13] Long-term effects of such treatment strategies have not been reported.
We previously documented preservation of lung function in asthmatic children in whom treatment was guided by AHR during a period of 2 years (CATO study clinical trial number NCT00158834 at clinicaltrials.gov).[14] This benefit was especially seen in those children who had low symptom scores and high levels of AHR. We hypothesized that the benefit of a treatment strategy that takes AHR into account would not persist beyond the duration of the intervention, because treatment would again be solely based on symptoms, and children may have bad perception. For this purpose we performed a prospective follow up study (clinical trial number NTC00441675 at clinicaltrials.gov) 3-7 years after the end of the CATO study.

METHODS

Patients

All patients who participated in the CATO study (n=206) were asked to take part 3-7 years after they finished CATO. At start of the CATO study, subjects were 6-16 y old and had moderately severe allergic asthma. They were symptomatic and/or had marked AHR.[14] Thus, there were 3 predefined subgroups, characterized by symptoms, AHR or both, and these were analyzed separately.[14] Subjects and/or parents (if children were younger than 18 years) gave written informed consent. The study was approved by the medical ethical committees of the participating centres.

Design

The mean interval between inclusion in the CATO study and follow-up was 4.4 years (range 3-7 years). Originally, patients were randomized and treated either on the basis of symptom scores (reference strategy) or on symptoms and AHR (AHR strategy)[14]. After the study, patients received usual care based on guidelines of the Dutch Pediatric Respiratory Group[15], largely corresponding to the GINA guidelines.[16]

Primary endpoint at follow-up was change in FEV_1 (%pred) from baseline at randomization. Secondary endpoints were PD_{20}, symptom scores and exacerbations, and ICS dose.

At follow-up, patients were seen twice. At the first visit, spirometry was performed (FEV_1, FVC and bronchodilator response 20 min after inhalation of 400 µg salbutamol; Masterscreen, Jaeger, Würzburg, Germany).[17] Data on the use of asthma medication were collected from pharmacists data records. An asthma exacerbation was defined as a deterioration of asthma requiring treatment with oral corticosteroids.[14] Data on oral corticosteroid prescription were collected from pharmacist records and patients or parents reports. On the second follow-up visit 6 weeks later, the % symptom-free days was determined, and a methacholine challenge test was performed using the same dosimeter protocol as in CATO.[14] Short acting β-agonists were stopped for 8 hours, and long-acting β-agonists for 36 hours before both visits.

Symptom scores

During the 6 weeks between the first and the second follow-up visit children recorded asthma symptoms twice daily on a diary card as used in CATO. Questions on cough, wheeze and shortness of breath were each scored on a 0-3 point scale, and the number of puffs of rescue β-agonists was recorded. A symptom-free day was defined as 24 hours with score 0 for cough, wheeze and shortness of breath.

Statistical analysis

Data at follow-up were compared with data of the same individuals during the CATO study period. As the use of age-specific reference equations for spirometry might affect the comparisons at follow-up that included children and young adults, we additionally analyzed FEV_1 normalized for a wide age range.[18] Comparison of categorical data was done with the Chi-square test. Continuous data were compared between or within groups using the Mann-Whitney and Wilcoxon test, respectively. For the evaluation of exacerbation rates, taking account of the duration of observation of individual patients, Poisson regression analysis was used. The highest dose of methacholine in the provocation tests was 1570 ug. If children had no 20% decrease of FEV_1 at this dose, the PD_{20} value was set at 1570 ug but was considered a right-censored observation, i.e. the true PD_{20} value will be larger than 1570 ug but remains unknown. Stata software (procedure Cnreg), which allows for such censored data, was used to evaluate the log-transformed PD_{20} outcomes. Profiles of changes of FEV_1 were calculated using repeated measurements Anova, which allows for occasional missing values. Correlation coefficients given are Spearman's. Data given are mean ± sem, unless indicated otherwise. P=0.05 (two-sided) was considered the limit of significance in all analyses.

RESULTS

One hundred and eighty-nine children and adolescents of the original CATO study population (n=206) were asked to participate, 17 could not be traced. One hundred thirty-seven children (67% of the original study population) participated in the first follow-up visit, 100 patients completed both visits. The main reasons for nonparticipation were lack of time and other priorities. The distribution of the participants over the original subgroups was the same for both studies (CATO and CATO follow up). The mean time interval between the last visit in the CATO study and the first follow-up visit was 4.4 years (range 3-7); Age and lung function of the population who participated differed significantly from those of the children who did not (Table 1).

Table 1 *- Characteristics of the children in the CATO-follow-up study and those who did not participate in the follow-up study, as measured at enrolment in the CATO[14] intervention study. Data given are numbers (%) of patients, mean (sd), or median (range). *at randomisation in the original Cato study ** upper limit of testing (1570 ug=highest dose)*

	Follow-up population n=137	No follow-up n=69	p-value
Gender (%)			
male	74 (54)	43 (62)	
female	63 (46)	26 (38)	0.32
Age at enrolment* (yrs)	10.4 (2.4)	11.8 (2.2)	<0.001
Age at follow-up (yrs)	16.8 (2.4)	18.2 (2.2)	<0.001
FEV_1 (%pred) at enrolment	98.7(14.9)	92(12.8)	0.002
FEV_1/FVC (%) at enrolment	82.7 (8.4)	80.4 (9.2)	0.17
PD_{20} (µg methacholine)	219	233	0.13
at enrolment	(0.8->1570)**	(0.8->1570)**	
% Symptom free days	47.3 (37.2)	50.6 (35.9)	0.48
at enrolment			
Treatment strategy (%)			
- AHR	76 (55)	28 (41)	
- Reference	61 (45)	41 (59)	0.06
Subgroup in CATO			
- AHR	58 (42)	33 (48)	
- Symptoms	32 (23)	14 (20)	
- AHR+symptoms	47 (34)	22 (32)	0.75
Drop-out during CATO study			
- Yes	5 (4)	16 (23)	
- No	132 (96)	53 (77)	<0.001

Lung function and AHR

FEV_1 and postbronchodilator FEV_1 were similar in children from the AHR and reference strategy groups (97.5% (sd 14.7) and 98.0% (15.7), p=0.69). Postbronchodilator FEV_1 was 105.9% (14.6) and 105.1% (16.0) for the AHR and reference strategy groups (p=0.76). FEV_1 changes from initial CATO baseline were not different between the treatment strategies: -1.6% (2.2) and -0.7% (1.7) for the AHR and reference strategy groups (p=0.37). FEV_1 changes since the end of the 2-years intervention were -1.4% (2.1) and +0.6% (1.6), respectively (figure 1A). Changes in FEV_1 at the first follow-up visit were similar for the 3 subgroups (figure 1B-D). A borderline-significant difference in change in FEV_1 was seen in the subgroup that had benefited most from the AHR strategy initially (figure 1B). Mean FEV_1 change since end of CATO study period in this subgroup was -6.0% (3.3) for the AHR strategy versus +1.7% (2.5) for the reference strategy (p=0.08). Repeated analysis using reference equations for a wider age range[18] produced similar results, with no significant differences in FEV_1 at follow-up between treatment strategies in the total population and in subgroups. No correlation was found between FEV_1 and time interval of follow-up visit or age at follow-up. A significant correlation between FEV_1 at start of the original CATO study and FEV_1 at follow-up existed (r=0.45, p<0.001). However, a lower FEV_1 did not correlate with FEV_1 decline.

***Figure 1** - Mean change from baseline of FEV_1 (%pred) during the 2 years CATO intervention study[14] in patients who completed the follow up study after the CATO trial, and at follow-up. Month 0 is time of enrolment, F.U. represents the follow-up assessment. Error bars represent standard errors and solid and open symbols depict the AHR and reference strategy group, respectively.*
A: total group of 137 patients
B: subgroup airway hyperresponsiveness, 58 patients
C: subgroup symptoms, 32 patients
D: subgroup with airway hyperresponsiveness + symptoms, 47 patients.

Figure 1A

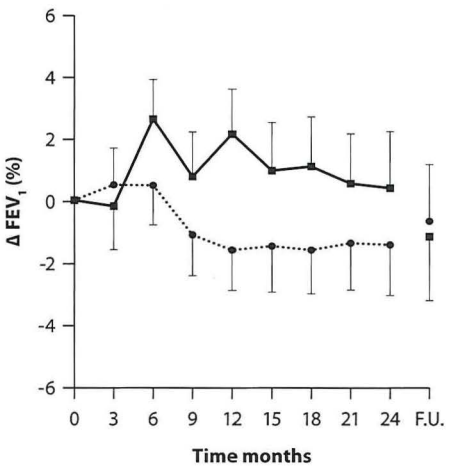


Figure 1B

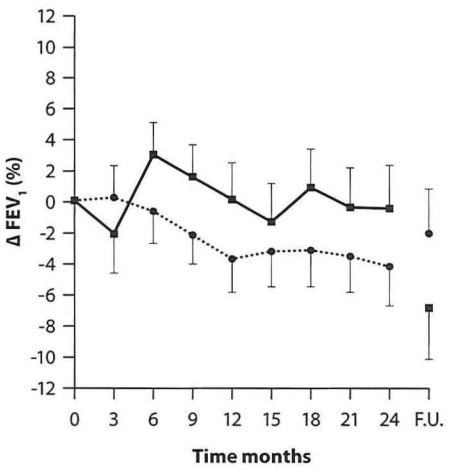


Figure 1C

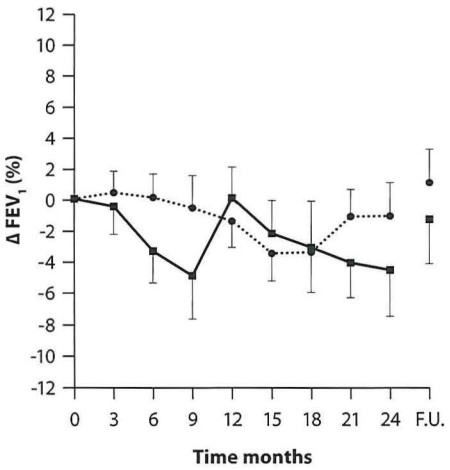
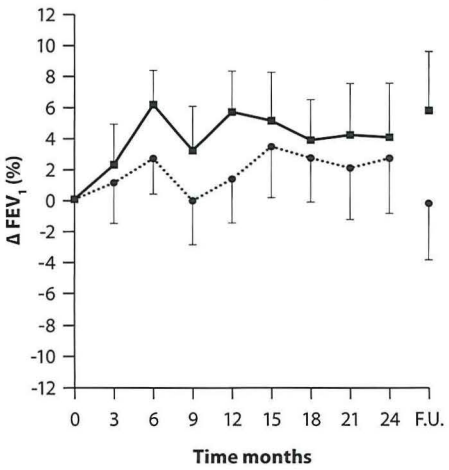
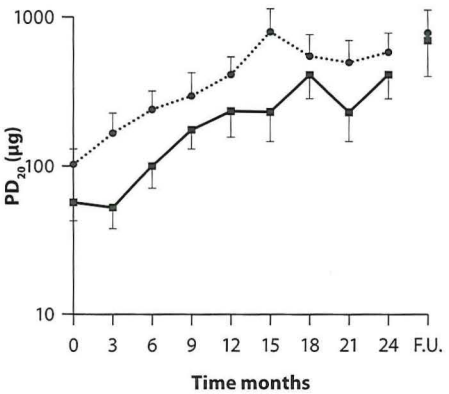


Figure 1D



PD_{20} at follow-up was assessed in 83 patients (36 from the AHR group and 47 from the reference strategy group). The increase of PD_{20} during the CATO study was maintained at follow-up for both treatment strategy groups (figure 2). No differences between treatment strategy arms and subgroups were found. The mean change from baseline of PD_{20} at CATO-FU was 3.3 and 2.8 doubling doses for the AHR and reference strategy, respectively (p=0.59).

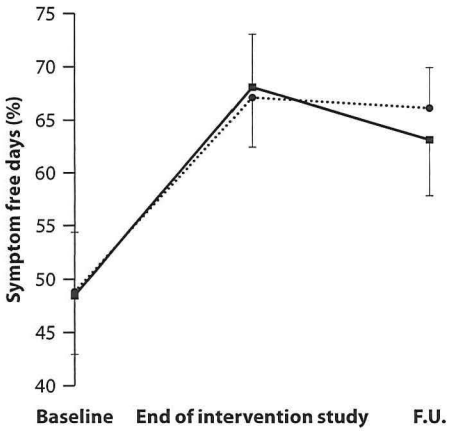
Figure 2 *- Geometric mean PD_{20} values during the 2 years CATO intervention study[14] in patients completing CATO-follow up study and at CATO-follow-up study according to treatment strategy. Month 0 is time of enrolment, F.U. represents the follow-up assessment. Error bars represent standard errors and solid and open symbols depict the AHR and reference strategy group, respectively. Error bars represent standard errors.*



Symptoms and exacerbations

Ninety patients returned evaluable diary cards (at least 20 completed days in the 6 weeks period). The mean % symptom-free days was 63 (5) and 67 (4) for the AHR and reference strategy groups, respectively (NS). The increase in % symptom-free days from CATO baseline was maintained at follow-up (figure 3).

Figure 3 *- Mean values of %symptom-free days at CATO[14] enrolment, at the end of the 2-years CATO intervention study in patients completing CATO-follow up study and at CATO-follow-up study. (FU) Error bars represent standard errors. Solid and open symbols depict the AHR- and reference strategy groups, respectively.*



During the CATO study 50 exacerbations requiring oral steroids occurred in 33 patients. During the follow-up years, 59 exacerbations were reported in 30 patients and another 4 exacerbations in 4 patients were reported between the 2 follow-up visits. The mean annual exacerbation rates were 0,26 in the year before- and 0.14 during the CATO study, and 0.10 in the follow-up years. The exacerbation rates during the intervention and follow-up years did not differ, and were significantly lower than before entering CATO. Baseline FEV_1 was inversely related to the exacerbation rate: per 10% points increase in FEV_1, the exacerbation rate decreased by 0.84 (p=0.03).

Medication use

At follow-up, all but 5 children reported to have asthma symptoms, although only 93 out of 137 (68%) still used ICS with a mean daily reported dose of 248 µg fluticasone equivalent. There was no difference in FEV_1, AHR or symptom scores in those with or without ICS at follow-up. The ICS dose, based on pharmacy registrations, gradually diminished over the years from 550 µg at the end of CATO to 235 µg at follow-up.

DISCUSSION

We prospectively assessed the long-term effects of 2 years of AHR-guided asthma treatment in children in whom we previously found a better evolution of lung function as a result of this intervention. After a mean interval of 4.4 years, range 3-7 years, the effect of AHR-guided treatment on lung function was lost. Overall, AHR and symptom-free days had remained at the same level as at the end of the 2-year intervention, despite the use of much lower doses of ICS and irrespective of the initial treatment strategy. We speculate that this reflects the natural history of asthma, which tends to improve during adolescence.[1] The subgroup of children with AHR and low symptom scores at baseline benefited most from the AHR strategy in the CATO study. This benefit was also lost at follow-up.

To our knowledge this is the first study which focuses on long-term effects of a phenotype-specific treatment strategy. Few studies have examined the persistence of effects of treatment strategies.[19] Recent data from the CAMP follow-up study showed that the positive effects of treatment with ICS on AHR, lung function and asthma control had disappeared 4 years after discontinuation of ICS.[20] Waalkens et al. reported similar findings already within 6 months after discontinuation of inhaled corticosteroids.[21] Contrary to the data of the CAMP follow-up study our data show that improvement in AHR and reduction of asthma exacerbations were maintained at the level reached during the intervention study, and we speculate that this is because of the continuous treatment with ICS. Contrary to the CAMP study the majority of children in our study continued their ICS.

That the subgroup with low symptom perception, that initially improved most from the AHR strategy, showed no lasting benefit beyond the duration of the intervention study is remarkable. One could imagine that participation in a 2-year intervention study with regular assessments of AHR and lung function might well improve symptom perception, and that this could specifically help children in the subgroup with low symptom scores despite AHR. This was apparently not the case, suggesting that there was no long-term effect of 2 years of AHR-guided treatment on symptom perception.

As can be expected in an adolescent population, a substantial number of the original study population could not be persuaded to co-operate in the follow-up. Our results may therefore have been biased by selection. Children in the follow-up study were younger and had higher FEV_1 values than those of the original study population. Adolescence, with a concomitant loss of interest and low priority of attending, may well have accounted for the younger age of those who participated at follow-up. We think it is unlikely that high FEV_1 values interact with the willingness to participate. However, as children who did not participate indeed had a lower baseline FEV_1, such an effect could have influenced our results[6]. It might be that children with lower FEV_1 benefited more from an intensive treatment strategy. It could be argued that expressing lung function as % predicted at follow-up becomes problematic when separate adult and paediatric reference equations for spirometry are used. We think that this has not affected our findings, as additional analyses using reference equations for a wide age range produced similar results.[18]

What could be the impact of our finding for clinical practice? The long-term evolution of lung function suggests that an AHR-guided treatment strategy may need to be sustained for longer than 2 years in order to preserve lung function. In view of the relatively low ICS doses at the end of the intervention, such a strategy would seem to be safe. It remains to be shown that such a strategy would be feasible and has the desired long-term effects.

In conclusion, we found that the beneficial effect on lung function of 2 years' asthma treatment guided by AHR was lost after 5 years of usual care, based on international guidelines. It remains to be shown if continuation of the experimental treatment strategy could have maintained the initial effect or might have further improved lung function.

REFERENCES

1. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003; 349: 1414-22.
2. James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, et al. Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. Am J Respir Crit Care Med 2005; 171: 109-14.
3. Strunk RC, Weiss ST, Yates KP, Tonascia J, Zeiger RS, Szefler SJ. Mild to moderate asthma affects lung growth in children and adolescents. J Allergy Clin Immunol 2006; 118: 1040-7.
4. Zeiger RS, Dawson C, Weiss S. Relationships between duration of asthma and asthma severity among children in the Childhood Asthma Management Program (CAMP). J Allergy Clin Immunol 1999; 103: 376-87.
5. Strachan D, Gerritsen J. Long-term outcome of early childhood wheezing: population data. Eur Respir J 1996; 21 Suppl: 42s-7s.
6. Roorda RJ. Prognostic factors for the outcome of childhood asthma in adolescence. Thorax 1996; 51 Suppl 1:S7-12.
7. Oswald H, Phelan PD, Lanigan A, Hibbert M, Carlin JB, Bowes G, et al. Childhood asthma and lung function in mid-adult life. Pediatr Pulmonol 1997; 23: 14-20.
8. Grol MH, Gerritsen J, Vonk JM, Schouten JP, Koeter GH, Rijcken B, et al. Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. A 30-year follow-up study. Am J Respir Crit Care Med 1999; 160: 1830-7.
9. van Essen-Zandvliet EE, Hughes MD, Waalkens HJ, Duiverman EJ, Pocock SJ, Kerrebijn KF. Effects of 22 months of treatment with inhaled corticosteroids and/or beta-2-agonists on lung function, airway responsiveness, and symptoms in children with asthma. The Dutch Chronic Non-specific Lung Disease Study Group [see comments]. Am Rev Respir Dis 1992; 146: 547-54.
10. van Rensen EL, Straathof KC, Veselic-Charvat MA, Zwinderman AH, Bel EH, Sterk PJ. Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. Thorax 1999; 54: 403-8.
11. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002; 360: 1715-21.
12. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med 1999; 159: 1043-51.
13. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. Am J Respir Crit Care Med 2005; 172: 831-6.
14. Nuijsink M, Hop WC, Sterk PJ, Duiverman EJ, de Jongste JC. Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial. Eur Respir J 2007; 30: 457-66.
15. Duiverman EJ, Brackel HJL, Merkus PJFM, Rottier BL, Brand PLP. Richtlijn "Astmabehandeling bij kinderen" van kinderlongartsen (2e herziening). II. Medicamenteuze behandeling. Ned Tijdschr Geneeskd 2003; 147: 1909-13.

16. GINA Report, Global Strategy for Asthma Management and Prevention. From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2012. http://www.ginasthma.org
17. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J 1993;16 Suppl: 5-40.
18. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates A, Pan H, Rosenthal M, Corey M, Lebeque P, Cole TJ. Reference ranges for spirometry across all ages. A new approach. Am J Respir Crit Care Med 2008; 177: 253-260.
19. Sinha IP, Williamson PR, Smyth RL. Outcomes in clinical trials of inhaled corticosteroids for children with asthma are narrowly focussed on short term disease activity. PLoS ONE 4: e6276. doi:10.1371/journal/pone.0006276
20. Strunk RC, Sternber AL, Szefler J, Zeiger RS, Bender B, Tonascia J, CAMP Research Group. Long-term budesonide or nedocromil treatment, once discontinued does not alter the course of mild to moderate asthma in children and adolescents. J Pediatr 2009; 154: 682-7.
21. Waalkens HJ, Van Essen-Zandvliet EEM, Hughes M, Gerritsen J, Duiverman EJ, Knol K. Cessation of long-term treatment with inhaled corticosteroid (budesonide) in children with asthma results in deterioration. Am Rev Respir Crit Care Dis 1993; 148: 1252-7

Chapter 4

Perception of bronchoconstriction: a complementary disease marker in children with asthma

Marianne Nuijsink, Wim C. J. Hop, Johan C. de Jongste, Peter J. Sterk, Eric J. Duiverman on behalf of the CATO Study Group

In press Journal of Asthma

4

4 Perception of bronchoconstriction: a complementary disease marker in children with asthma

ABSTRACT

Introduction

Asthma guidelines use symptoms as the most important aspect of asthma control. Symptom perception varies widely between individuals. Over-perception as well as underperception of bronchoconstriction could have a negative effect on asthma management. We hypothesized that perception of bronchoconstriction in childhood asthma is not related to common measures of disease control. For that reason we examined the clinical determinants of the perception of bronchoconstriction and the repeatability of perception measurements.

Patients and methods

In school-age children with moderately severe atopic asthma we measured perception of bronchoconstriction (decrease in forced expiratory volume in 1 second (FEV_1)) during methacholine bronchoprovocation challenges. The perception of bronchoconstriction was assessed as the slope of the relation between FEV_1 and Borg score, and as the Borg score at 20% decrease in FEV_1 from baseline during the provocation test (PS_{20}). Data from subjects who had a 20% or more decrease in FEV_1 (n=112) were used for analysis. Fifty-four children repeated the test after 3 months. Symptoms, use of rescue medication and peak expiratory flows were scored in diaries during 2 weeks before testing.

Results

Symptom perception was significantly better in children without ($PD_{20} > 1570$ µg, n=28) than in children with airway hyperresponsiveness ($PD_{20} \leq 1570$ µg, n=112), slope 0.22 versus 0.13 respectively ($p<0.001$). Borg scores correlated with PD_{20} ($p=0.01$), baseline FEV_1 (only for slope, $p=0.04$) and use of rescue beta agonist ($p=0.01$), but not with other aspects of asthma control. Repeatability of Borg scores was good (slope: $R=0.59$, PS_{20}: $R=0.52$)

Conclusion

Poorer symptom perception in asthmatic children correlated with hyper-responsiveness, and was associated with lower baseline FEV_1 and less use of rescue bronchodilators. This suggests that measurement of symptom perception should be taken into account in individual management plans for children with asthma.

INTRODUCTION

Perception of bronchoconstriction is an important factor in asthma management. According to the GINA guidelines the treatment of childhood asthma should be based on the level of control, which is largely estimated from reported symptoms and objective lung function measures.[1] Inadequate perception of bronchoconstriction might therefore lead to under- or overtreatment. It has been shown that diminished perception of bronchoconstriction is related to more severe asthma and fatal asthma attacks in adults and children.[2-7] Asthma medication and the duration of asthma seem to influence the adequacy of perception in asthmatics.[3,8-10] Although several studies examined risk factors for poor perception in adult asthma patients, only a few studies have been performed in children. Baker et al studied perception of airway obstruction in 35 children with asthma, and found poor perception in approximately half of the children. They found no correlation with baseline FEV_1 or with airway hyperresponsiveness (AHR).[11] In contrast, Motomura showed that children with severe AHR had a lower perception of bronchoconstriction than children with mild AHR.[12] Results of repeated measurement of perception during exercise testing showed poor repeatability.[13] The Borg score was developed to assess the perception of dyspnea and can be used during a bronchial provocation test to assess the perception of bronchoconstriction in a standardized way.[14] The aim of this study was to define clinical parameters that are risk factors for poor perception of bronchoconstriction in children with stable, moderately severe atopic asthma. In addition, we examined whether measurement of perception was repeatable.

METHODS

Patients

The present study was performed within the CATO (Children Asthma Therapy Optimal) study, a multi-centre, double blind, parallel group randomised 2-year intervention trial. Details of this study have been published.[15] Briefly, 210 children (6-16 y) with moderate atopic asthma were included, selected on the basis of symptom scores and/or presence of AHR. The subjects were recruited from 15 paediatric clinics, including 7 university hospitals, in the Netherlands. Every 3 months, children kept a symptom score diary for two weeks and underwent lung function and methacholine challenge tests. Medication was adjusted according to algorithms based on symptom score or on AHR and symptom score. All the children used fluticasone (range 100-1000 µg/d) and n=73 used salmeterol (100 µg/d).

Design

Borg scores were measured at the time that a substantial part of the subjects had already completed the CATO study. For that reason a Borg score was measured in 140 children. One hundred and twelve did reach a PD_{20} methacholine, data of those children were used for analysis. Fifty-four repeated the test 3 months later.

Perception was expressed as the slope of the relation between changes in FEV_1 and Borg scores, and as the Borg score at a 20% fall in forced expiratory volume in 1 sec (PS_{20}). All parents and children >12 years gave written informed consent. The study was approved by the medical ethics committees of all participating hospitals.

Diary Cards

Twice daily the subjects filled in a diary card during 2 weeks before a clinic visit. Symptoms of asthma (cough, shortness of breath and wheezing) during night and day were recorded on a 4 point scale (0 (=no symptoms) to 3 (=severe symptoms interfering with activity or sleep) and the use of salbutamol inhalations for rescue therapy was recorded. Symptoms were scored as the percentage of days in 2 weeks with at least 1 symptom recorded. Use of rescue medication was expressed as the percentage of days out of 2 weeks with at least 1 dose of salbutamol. Triplicate measurements of peak expiratory flow (PEF) were performed in the morning and evening, before medication. Diurnal PEF variability was defined as the mean value of 2 weeks calculated from the highest minus the lowest daily value, divided by the mean.[16]

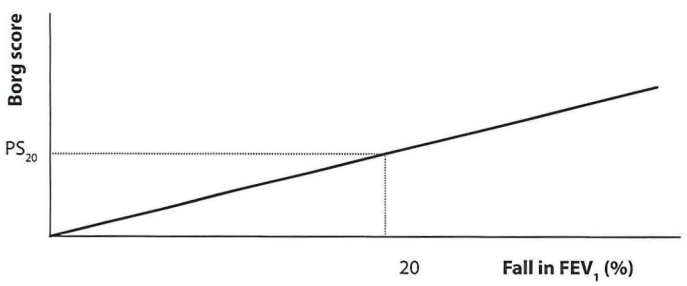
Lung function and AHR

Study medication was withheld for 36 hours and short-acting β-agonists for 8 hours prior to lung function and methacholine challenge testing. FEV_1 and forced vital capacity (FVC) were measured using a heated pneumotachograph. Values are reported as percentage of predicted. AHR was tested by methacholine challenge using a dosimeter.[17] Nebulised methacholine bromide was given in doubling concentrations (0.076 mg/mL – 78.5 mg/mL). Three minutes after each inhalation FEV_1 was measured. The provocation dose causing a 20% fall in FEV_1 from baseline (PD_{20}) was calculated by linear interpolation of the last two points of the log dose response curve where FEV_1 had fallen below 20% of baseline value. At the end of the challenge test 800 µg salbutamol was administered to reverse bronchoconstriction and FEV_1 was measured again.

Assessment of perception of dyspnea

During the challenge test the severity of dyspnea was assessed by a Borg scale.[14] The Borg scale is a vertical list with labeled categories: 0 (no breathlessness at all) – 10 (most extreme breathlessness ever experienced). After each dose step methacholine children were asked: "How severe is your breathlessness during and directly after this inhalation?" For each child an individual 'Borg slope' (derived from Borg scores plotted against % fall in FEV_1 from baseline) and the Borg score at 20 percent fall in FEV_1 (PS_{20}) (figure 1) were calculated. During the test the patient was blinded to the lung function response.

Figure 1 *- Schematic representation of calculation of PS_{20}. The PS_{20} was found by linear interpolation of Borg scores (vertical axis) at the point where a 20% fall in FEV_1 during methacholine challenge was detected (horizontal axis).*



Statistical analysis

All variables with a non-Gaussian distribution (symptom score, PEF-variability and PD_{20}) were lognormalized. Data are given as medians (p10 - p90). Borg slope was calculated for 28 subjects without AHR (PD_{20}> 1570 ug). Borg-slope and PS_{20} were calculated for 112 subjects with a PD_{20}<1570 µg. Borg slope was calculated by linear regression. PS_{20} was determined by interpolation of the 2 last perception scores, before and after the 20 percent fall in FEV_1. The relationship between Borg slopes and PS_{20} and asthma symptom scores, PEF-variability, usage of rescue medication, FEV_1 (%pred), PD_{20} methacholine and age were calculated using Spearman's rank correlation coefficients. A two-sided p value of <0.05 was considered statistically significant.

RESULTS

We measured Borg scores in n=140 children. Twenty eight of them were not hyperresponsive (PD_{20}>1570 µg). In these children the median maximal fall in FEV_1 at bronchoprovocation testing was 14.6% (-17.7 to -3.6%). The median Borg slope was 0.22 (0.04 to 0.43). Baseline characteristics of all children with AHR are summarized in table 1.

Table 1 *- Baseline characteristics of the study population. All data are median [10-90 percentile]. PD_{20}: provocative dose of methacholine causing a 20% fall in FEV_1 from baseline. Symptom score expressed as the percentage days, out of 2 weeks, with at least one symptom recorded. Use of rescue medication expressed as the percentage of days, out of 2 weeks, with at least 1 dose salbutamol. PEF-variability: highest PEF minus lowest PEF divided by the mean PEF. Borg slope: relation between change in FEV_1 (%) and Borg score. PS_{20}: Borg score at a 20% fall in FEV_1 from baseline.*

Age (years)	11.8 [9.0 - 15.6]
Baseline FEV_1 (%predicted)	98 [79 – 116]
PD_{20} µg	154 [26 – 1022]
Symptom score (%)	20 [0 – 100]
Use of rescue medication (%)	0 [0 – 62]
PEF variability (%)	4.7 [1.8 – 10.0]
Borg slope	0.13 [0.04 – 0.26]
PS_{20}	3.5 [1.0 – 7.0]

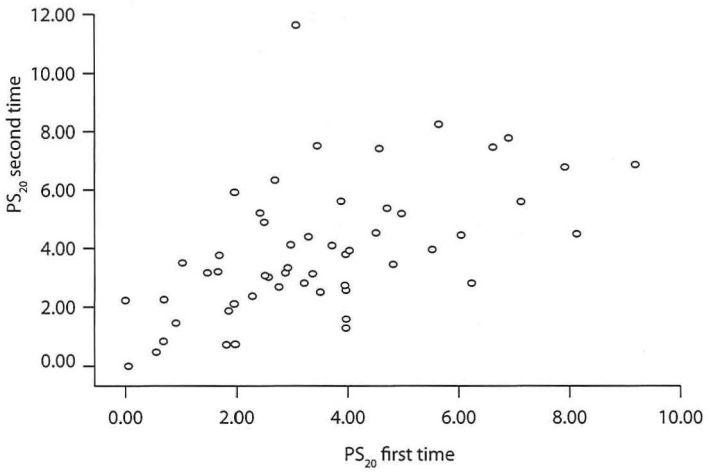
At bronchoprovocation testing, the median maximal fall in FEV_1 from baseline was 23.0% (20.3 to 32.1). The median Borg score was 0 (0-2) before, and 4 (1-8) after methacholine challenge. After salbutamol, the median Borg score was 0.0 (0-2). The median Borg slope was 0.13 (0.04-0.26) and the median PS_{20} value was 3.5 (1.0-7.0). The age of the child, duration of asthma, the dose of inhaled corticosteroids and the use of salmeterol did not correlate with the Borg slope or PS_{20}. Significant associations were present between baseline FEV_1 and Borg slope (Rs=0.20, p=0.04), between PD_{20} and Borg slope (Rs=0.24, p=0.01) and between PD_{20} and PS_{20} (Rs=0.26, p=0.01). A significant positive correlation existed between PS_{20} and the use of rescue salbutamol (Rs=0.26, p<0.01). No relation was found between symptom severity or PEF variability and Borg slope or PS_{20} (Table 2).

Table 2 *- Correlation of clinical markers of asthma control, lung function and AHR versus Borg scores (slope and PS_{20}). Data are Spearman rank correlation coefficients. Borg slope: relation between change in FEV_1 and Borg score. PS_{20}: Borg score at a 20% fall in FEV_1 from baseline. Symptom score: percentage days, out of 2 weeks, with at least 1 symptom recorded. Use of rescue medication: the percentage of days, out of 2 weeks, with at least one dose salbutamol. PEF-variability: highest PEF minus lowest PEF divided by the mean PEF. PD_{20}: provocative dose of methacholine causing a 20% fall in FEV_1 from baseline.*

	Borg slope		PS_{20}	
	Rs	p	Rs	p
Diary card data				
Symptoms	-0.51	0.60	0.11	0.25
Salbutamol use	0.12	0.20	0.26	0.01
PEF-variability	0.00	0.99	0.07	0.18
Baseline FEV_1	0.20	0.04	0.13	0.18
PD_{20}	0.24	0.01	0.26	0.01

The correlation coefficients between Borg slope and PS_{20} was 0.82 (p<0.001). There was good agreement between Borg slopes at the first and second occasion (R=0.59, p<0.001, n=54) and PS_{20} (R=0.52, p<0.001, n=54)(Figure 2).

Figure 2 *- Relation between Borg score at first and second occasion. PS_{20}: Borg score at a 20% fall in FEV_1 from baseline.*



DISCUSSION

In this study we examined symptom perception in relation to measures of asthma control in school-age children with moderately severe asthma. We aimed to identify risk factors for impaired symptom perception, as these could alert for undertreatment. Our results show that appropriate perception of bronchoconstriction is less likely in case of more severe AHR and lower baseline FEV_1, and we found a positive correlation between perception and use of rescue medication. No relation was present between symptom scores and perception. Perception could be measured reproducibly.

Panditi et al. reported on the reproducibility of Borg ratings over time but this was only tested in a single population of asthmatic children during repeated exercise testing.[13] The period between the consecutive tests in this study was 8 weeks. No significant correlation between the two Borg ratings over time was found. The authors of this study suggested that this could be due to the fact that confounders such as AHR were not analyzed. In our study, few factors appeared to be related to symptom perception, and these were AHR and baseline lung function. Conflicting data on the relation between AHR and perception have been reported in adults as well as in children with asthma. In a small group of 35 asthmatic children poor perception was not associated with airway hyperreactivity.[11] In contrast, in a large population of Japanese children AHR

was related to perception of bronchoconstriction, in agreement with our findings.[12] The difference might be explained by sample size. Our finding suggests that children with more severe AHR would be at risk for undertreatment. This is in line with our previous finding that asthmatic children who have few symptoms in combination with more severe AHR are at risk for an unfavorable clinical course of their asthma, with more rapid decline in lung function compared to those who reported more symptoms.[15] Also, low baseline lung function was correlated with lower symptom perception. In this line, Van Gent et al. recently reported that children with AHR without diagnosed asthma perceive induced bronchoconstriction less well than those with diagnosed asthma.[18] Concerning the relation between baseline lung function and AHR, conflicting data are reported in children as well as in adults.[11,20]

Interestingly, we found that low use of rescue medication was associated with lower symptom perception, in a dose-dependent manner. This supports earlier speculation that underestimation of asthma symptoms predisposes to severe asthma attacks in adult asthmatics and children.[3,4,6,19]

We attempted to separate the sensitivity and dose-response characteristics of the Borg score. To that end we analyzed the slope of the relation between FEV_1 and Borg score, and the scores at a given level of bronchoconstriction (PS_{20}). It appeared that both ways of expressing symptom perception were closely correlated, so as it seems analyzing one of the two would be sufficient. For ease of calculation we would recommend the PS_{20}.

What are the clinical implications of our findings? Wide intersubject variability in perception of changes of airway diameter is reported in children and adults with asthma.[11] Symptom perception is associated with functional morbidity, and poor perception with near fatal asthma attacks.[4-7,21] In the present GINA guidelines, the level of asthma control does not involve a measure of symptom perception or hyperresponsiveness. We think that there is sufficient evidence that AHR is an important determinant of morbidity and future risk, and appears related to poor symptom perception.[22] Hence, measurement of perception of dyspnea provides potentially useful complementary information compared to common clinical disease markers. We propose that AHR should be assessed in settings where this is practically feasible to detect children at risk for more severe disease and under-treatment.[15] Adding a Borg score to the routine protocol of such a bronchial challenge test could help to discover impaired symptom perception and thereby allow improved asthma management.

In conclusion, we found that poor symptom perception in asthmatic children correlated with increased AHR, lower FEV_1 and was associated with less use of rescue bronchodilators. Perception was not related to other aspects of asthma control. This indicates that perception is a complementary disease marker in children with asthma. We propose that assessment of Borg scores could be a useful addition to routine bronchoprovocation testing in children.

REFERENCES

1. Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2011. Available from: http://www.ginasthma.org/
2. Veen JC, Smits HH, Ravensberg AJ, Hiemstra PS, Sterk PJ, Bel EH. Impaired perception of dyspnea in patients with severe asthma. Relation to sputum eosinophils. Am J Respir Crit Care Med. 1998; 158:1134-41
3. Bijl-Hofland ID, Cloosterman SG, Folgering HT, van den Elshout FJ, van Weel C, van Schayck CP. Inhaled corticosteroids, combined with long-acting beta(2)-agonists, improve the perception of bronchoconstriction in asthma. Am J Respir Crit Care Med. 2001;164:764-9
4. Kikuchi Y, Okabe S, Tamura G, Hida W, Homma M, Shirato K, Takishima T. Chemosensitivity and perception of dyspnea in patients with a history of near-fatal asthma. N Engl J Med. 1994;330:1329-34
5. Magadle R, Berar-Yanay N, Weiner P. The risk of hospitalization and near-fatal and fatal asthma in relation to the perception of dyspnea. Chest. 2002;121:329-33
6. Kifle Y, Seng V, Davenport PW. Magnitude estimation of inspiratory resistive loads in children with life-threatening asthma. Am J Respir Crit Care Med. 1997;156:1530-5
7. Turner MO, Noertjojo K, Vedal S, Bai T, Crump S, Fitzgerald JM. Risk factors for near-fatal asthma. A case-control study in hospitalized patients with asthma. Am J Respir Crit Care Med. 1998;157:1804-9.
8. Boulet LP, Turcotte H, Cartier A, Milot J, Cote J, Malo JL, Laviolette M. Influence of beclomethasone and salmeterol on the perception of methacholine-induced bronchoconstriction. Chest. 1998;114:373-9
9. Salome CM, Reddel HK, Ware SI, Roberts AM, Jenkins CR, Marks GB, Woolcock AJ. Effect of budesonide on the perception of induced airway narrowing in subjects with asthma. Am J Respir Crit Care Med. 2002;165:15-21.
10. Salome CM, Leuppi JD, Freed R, Marks GB. Perception of airway narrowing during reduction of inhaled corticosteroids and asthma exacerbation. Thorax. 2003;58:1042-7
11. Baker RR, Mishoe SC, Zaitoun FH, Arant CB, Lucas J, Rupp NT. Poor perception of airway obstruction in children with asthma. J Asthma. 2000;37:613-24
12. Motomura C, Odajima H, Tezuka J, Harada J, Okada K, Nishima S. Perception of dyspnea during acetylcholine-induced bronchoconstriction in asthmatic children. Ann Allergy Asthma Immunol. 2009;102:121-4
13. Panditi S, Silverman M. Perception of exercise induced asthma by children and their parents. Arch Dis Child. 2003;88:807-11
14. Wilson RC, Jones PW. A comparison of the visual analogue scale and modified Borg scale for the measurement of dyspnoea during exercise. Clin Sci (Lond). 1989;76:277-82
15. Nuijsink M, Hop WC, Sterk PJ, Duiverman EJ, de Jongste JC. Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial. Eur Respir J. 2007;30:457-66
16. Higgins BG, Britton JR, Chinn S, Lai KK, Burney PG, Tattersfield AE. Factors affecting peak expiratory flow variability and bronchial reactivity in a random population sample. Thorax. 1993;48:899-905

17. 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;45:199-202.
18. van Gent R, van Essen-Zandvliet LE, Rovers MM, Kimpen JL, de Meer G, van der Ent CK. Poor perception of dyspnoea in children with undiagnosed asthma. Eur Respir J. 2007;30:887-91
19. Julius SM, Davenport KL, Davenport PW. Perception of intrinsic and extrinsic respiratory loads in children with life-threatening asthma. Pediatr Pulmonol. 2002;34:425-33
20. Boulet LP, Leblanc P, Turcotte H. Perception scoring of induced bronchoconstriction as an index of awareness of asthma symptoms. Chest. 1994;105:1430-3.
21. Feldman JM, McQuaid EL, Klein RB, Kopel SJ, Nassau JH, Mitchell DK, Wamboldt MZ, Fritz GK. Symptom perception and functional morbidity across a 1-year follow-up in pediatric asthma. Pediatr Pulmonol. 2007;42:339-47
22. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, de Jongste JC, Kerstjens HA, Lazarus SC, Levy ML, O'Byrne PM, Partridge MR, Pavord ID, Sears MR, Sterk PJ, Stoloff SW, Sullivan SD, Szefler SJ, Thomas MD, Wenzel SE. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med. 2009;180:59-99

Chapter 5

Urinary Eosinophil Protein X in children with atopic asthma

M Nuijsink, W C J Hop, P J Sterk, E J Duiverman, P S Hiemstra, J C de Jongste on behalf of the CATO Study Group

Mediators of Inflammation 2007

5

5 Urinary Eosinophil Protein X in children with atopic asthma

ABSTRACT

The aim of this study was to investigate the relationship between urinary eosinophil protein X (uEPX) and asthma symptoms, lung function and other markers of eosinophilic airway inflammation in asthmatic schoolchildren.

Methods

A cross-sectional study was performed in 180 steroid dependent atopic children with stable, moderately severe asthma, who were stable on 200 or 500 µg of fluticasone per day. uEPX was measured in a single sample of urine and normalised for creatinine concentration (uEPX/c). Symptom scores were kept on a diary card. FEV_1 and PD_{20} methacholine were measured. Sputum induction was performed in 49 and FE_{NO} levels measured in 24 children.

Results

We found an inverse correlation between uEPX/c and FEV_1 ($r = -0.20$, $p = 0.01$) and a borderline significant correlation between uEPX/ c and PD_{20} methacholine ($r = -0.15$, $p = 0.06$). Symptom score, %eosinophils and ECP in induced sputum and FE_{NO} levels did not correlate with uEPX/c.

Conclusion

uEPX/c levels did not correlate with established markers of asthma severity and eosinophilic airway inflammation in atopic asthmatic children.

INTRODUCTION

Eosinophilic airway inflammation is the pathological substrate of allergic asthma both in adults and in children.[1,2] The severity of airway inflammation correlates poorly with symptoms and lung function.[3] As asthma treatment with inhaled steroids aims at reducing inflammation, there is a need to monitor the disease with a marker of inflammation.[4,5] Potential markers are serum eosinophilic cationic protein (ECP), induced sputum cellularity and soluble markers and the concentration of nitric oxide in exhaled air (FE_{NO}).[6-8]

Eosinophil protein X (EPX) is one of the toxic proteins present in eosinophil granules and is released by activated eosinophils. EPX can be measured accurately in urine (uEPX).[9] Therefore, uEPX can be regarded as a marker of eosinophil degranulation in vivo.[10] uEPX levels in allergic asthmatic children were found to be significantly higher than in healthy controls.[11-14] Treatment with inhaled steroids reduced uEPX levels.[14] We hypothesised that measuring EPX in urine could potentially prove useful for monitoring eosinophilic airway inflammation in children and may complement other markers of asthma control such as symptom scores and lung function

The aim of this study was to evaluate the relationship between uEPX and current symptoms and lung function parameters, and the relation between uEPX, induced sputum eosinophilia and FE_{NO}. For this purpose we analysed cross-sectional data obtained at enrolment for a multi-centre trial.

METHODS

Subjects

Data were obtained from steroid-dependent asthmatic children who took part in a large randomised controlled multi-centre trial (CATO: Children Asthma Therapy Optimal). One hundred and eighty atopic (RAST ≥ class 1 for at least one airborne allergen) children, median age 10.3 years (range 6–16 years), with a documented clinical history of moderately severe asthma were recruited from paediatric clinics in 8 general hospitals and 7 university hospitals in The Netherlands. All had been treated with inhaled corticosteroids (ICS) for at least 4 weeks. Data were obtained during a clinic visit at the end of the run in period (4-12 weeks). During this period they were treated with fluticasone dipropionate 200 µg/d (n=102) or 500 µg/d (n=78). All parents and children if > 12 years gave their written informed consent. The study was approved by the medical ethics committees of all participating hospitals.

Symptom scores

Two weeks before visiting the hospital patients kept a diary in which symptoms (shortness of breath, wheeze and cough) were scored twice a day each on a 4 point (0-3) scale. Cumulative symptom scores were calculated over 14 days (maximum score 252).

Fractional exhaled nitric oxide

The fractional concentration of exhaled nitric oxide (FE_{NO}) was measured with the on-line single breath method, using the NIOX NO-analyser (Aerocrine, Stockholm, Sweden) according to ERS/ATS guidelines.[15] As FE_{NO} could only be measured in 1 participating university centre, only part of the children underwent FE_{NO} measurements.

Flow-volume curves

Flow-volume curves and forced expiratory volume in 1 second (FEV_1) were measured on a dry rolling seal spirometer according to recommendations.[16] Results are expressed as percentage of predicted values.[17]

Bronchial challenge test

Bronchial responsiveness was determined by a methacholine challenge.[18] PD_{20}methacholine was assessed by linear interpolation of the last two points of the log dose response curve where FEV_1 had fallen below 20% of baseline value.

Sputum induction and processing

Sputum induction was performed by 5 university centers and 3 pediatric clinics in general hospitals. Sputum was induced according to a standardized method by inhaling an aerosol prepared from hypertonic sodium chloride 4.5% w/v.[19,20] Differential cell counts of the cytospins were performed by counting 500 cells. Sputum samples containing more than 80% squamous cells were excluded from the analysis.[20]

In sputum supernatant, ECP was measured by fluoro-enzyme immuno-assay (Pharmacia, Uppsala, Sweden)

Urinary Eosinophil Protein X

A spot sample urine was collected from each individual at the clinic visit and immediately stored at -20° C. uEPX was determined using a commercial enzyme-linked immunosorbent assay (ELISA) for human EPX in 50-fold diluted samples according to the manufacturers recommendations (Medical and Biological Laboratories, Naka-ku Nagoya, Japan). The sensitivity of the assay was 0.62 ng/ml. Urinary creatinine levels were measured by using the alkaline picrate method (Jaffé reaction) (Roche, Mannheim, Germany). Urinary EPX concentrations were expressed as µg per mmol creatinine (uEPX/c).

Data analysis

All variables with a non-Gaussian distribution (symptom score, PD_{20}methacholine, FE_{NO}, % eosinophils in sputum, ECP in sputum and uEPX) could be normalized by log-transformation. The significance of the relation between uEPX and lung function variables or other markers of inflammation was calculated using Spearman's rank correlation coefficients. A two-sided p value of <0.05 was considered statistically significant.

RESULTS

One hundred and eighty subjects (105 (58.3%) boys) participated. Asthma was controlled by fluticasone dipropionate 200 µg/day (n = 102) or 500 µg/day (n = 78).

All subjects performed spirometry and recorded symptoms in a diary. Six children inhaled short acting β agonists prior to the visit, their results were excluded from analysis. One hundred and seventy eight children performed a bronchial challenge test; two had FEV_1 <80% of personal best and were therefore not tested. Children who had used β-agonist within 8 hours before the test (n = 6) were again excluded. For logistic reasons sputum induction was done in part of the subjects. Forty-nine of the 98 sputum inductions yielded adequate sputum samples (50%). At randomisation, only one university centre had the facility to measure FE_{NO} (n = 24 subjects).

Baseline results of lung function, symptom score and markers of inflammation are given in table 1. uEPX/c showed a log-normal distribution, median 185 µg/mmol creatinine (range 2–3114 µg/mmol creatinine). UEPX/c did not correlate with age and was not different between boys and girls.

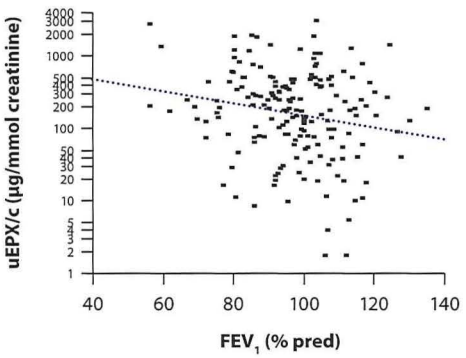
Table 1 *- Characteristics of study subjects Values are median (range). FEV_1: forced expiratory volume in 1 second; PD_{20}methacholine: provocative dose of methacholine causing FEV_1 fall 20% from baseline; ECP: eosinophil cationic protein; FE_{NO}: fractional concentration of nitric oxide in exhaled air; uEPX/c, urinary eosinophil protein X per mmol creatinine.*

	Fluticasone dose		Total
	200 µg/day	**500 µg/day**	
Age (years)	10.0 (96.4-16.8)	11.3 (6.4-16.7)	10.3 (6.4–16.8)
	n=102	n=78	n=180
Gender (m/f)	60/40	45/33	105/75
FEV_1 (%pred)	99 (56-135)	96 (56-96)	97 (56–135)
	n=101	n=73	n=174
Cumulative symptom score	18.5 (0-113)	14 (0-152)	17.0 (0–152)
	n=102	n=78	n=180
PD_{20}methacholine (µg)	200 (3->1570)	48 (1->1570)	68 (1 –>1570.0)
	n=100	n=72	n=172
Eosinophils sputum (%)	1 (0-72)	1 (0-43)	1.0 (0–72)
	n=29	n=20	n=49
ECP sputum (ng/ml)	17 (0-2345)	38 (0-538)	29 (0–2345)
	n=24	n=19	n=43
FE_{NO} (ppb)	11 (5-63)	9 (1-29)	10 (1–63)
	n=12	n=12	n=24
uEPX/c (µg/mmol)	189 (2-2828)	180 (10-3114)	185 (2-3114)
	n=102	n=78	n=180

Relation between uEPX/c and clinical markers of asthma severity (table 2)

uEPX/c did not correlate with symptom scores or inhaled steroid dose. There was a significant inverse correlation of uEPX/c with FEV_1 ($r = -0.18$, $p = 0.02$) (figure 1).

Figure 1 *- Scatterplot of urinary eosinophil protein X per mmol creatinine (uEPX) versus forced expiratory volume in 1 second (FEV_1) n=174*



The association between uEPX/c and FEV_1 did not significantly differ between children using 200 µg fluticasone per day and those using 500 µg (Anova $p = 0.19$). For each 10 percentage points increase of FEV_1 (%pred) the geometric mean EPX/c ratio decreases 18% (95% CI: 5,30%). The correlation between uEPX/c and PD_{20}methacholine was borderline significant ($R = -0.14$, $p = 0.08$).

Relation between uEPX and markers of asthmatic airway inflammation (table 2)

uEPX/c did not correlate with the %eosinophils or ECP in induced sputum, or with FE_{NO}. Relations between uEPX and PD_{20}methacholine or markers of asthmatic airway inflammation did not significantly differ when analysis was adjusted for fluticasone dose.

Correlations were similar when children with eczema were excluded from the analysis.

Table 2 *- Correlations between uEPX-c and clinical markers of asthma severity or markers of asthmatic inflammation. R values were all analyzed by Spearman's rank correlation test. uEPX/c: urinary eosinophil protein X per mmol creatinine; FEV_1: forced expiratory volume in 1 second; PD_{20}methacholine: provocative dose of methacholine causing FEV_1 fall 20% from baseline; ECP: eosinophil cationic protein; FE_{NO}: fractional concentration of nitric oxide in exhaled air.*

Variable	N	Log uEPX/c	
		R	P
Age	180	-0.01	0.90
Symptom score	180	0.03	0.72
FEV_1	174	-0.18	0.02
PD_{20}methacholine	172	-0.14	0.08
%Eosinophils In Sputum	49	0.17	0.26
ECP Sputum	43	-0.03	0.83
FE_{NO}	24	0.16	0.46

DISCUSSION

We found a significant correlation of uEPX/c and FEV_1, and no association between uEPX/c and bronchial responsiveness or symptom scores in a large group of children with moderately severe allergic asthma. In subgroups, no significant correlations between uEPX/c and other markers of eosinophilic airways inflammation (% eosinophils and ECP in induced sputum or FE_{NO}) were found.

This is the first study reporting uEPX/c levels in relation with markers of asthma severity and inflammation in a large population of children with atopic asthma, treated with inhaled steroids. Lugosi et al. has shown that uEPX levels were increased in symptomatic versus non-symptomatic children with asthma, treated with inhaled steroids or disodium cromoglycate, and Oosaki et al. found significantly elevated uEPX levels during acute asthma exacerbations in children.[21,22] All subjects included in our study had stable well controlled asthma, as evidenced by a median cumulative symptom score of only 17 of a maximum of 252. Conflicting data have been published on the association between uEPX/c and pulmonary function tests.[21,23] We found a significant negative correlation between FEV_1 and uEPX/c. It should be mentioned that the scatter was wide and individual uEPX/c therefore varied widely for a given FEV_1 level. Hence, such correlations are unlikely to be detected in smaller

groups. However, the within-subject variation of both parameters in time had not been studied. We confirmed our hypothesis that uEPX/c and bronchial hyperresponsiveness are not closely correlated. Lack of correlation between the severity of bronchial hyperresponsiveness and uEPX/c levels was also reported in 3 previous studies.[24-26] A close correlation between bronchial hyperresponsiveness and uEPX/c was not expected, because bronchial hyperresponsiveness is multifactorial and not only caused by (eosinophilic) airways inflammation, but also by airway geometry, airway remodeling and autonomic dysregulation.

Our hypothesis that uEPX/c would correlate with markers of eosinophilic airway inflammation could not be confirmed, as we found no correlation between uEPX/c and the percentage eosinophils in induced sputum. Others likewise found no correlation between uEPX/c and bronchoalveolar lavage cell counts in adult asthmatic patients.[10] An alternative explanation for not finding significant correlations between percentages of sputum eosinophils and uEPX/c could be that uEPX is only released by activated eosinophils, whereas in sputum we counted activated as well as non-activated eosinophils. Also, the numbers of children from whom suitable sputum samples or FE_{NO} values were obtained was relatively small.

We found no correlation between uEPX/c and sputum ECP levels. In contrast, Mattes et al. reported a positive correlation between uEPX/c and sputum ECP in 25 stable asthmatic children on inhaled corticosteroids.[11] They found much higher ECP concentrations than we did (median 453 ng/ml, range 40 – 2600 and 29 ng/ml, 1-2345 respectively). The reason for this is not clear, but may relate to different sputum processing techniques.

One could argue that the lack of correlation between uEPX/c and percentage of sputum eosinophils, or ECP levels in sputum supernatant, could be due to the wide scatter of uEPX. However all urine samples were immediately stored at -20 °C and uEPX and urinary creatinine levels measurements were performed in a central laboratory (Leiden University Medical Hospital) to reduce variability in the analysis. All EPX measurements were done in duplicate and the within-subject reproducibility of uEPX levels was good.

It has been reported that in atopic dermatitis concentrations of eosinophil specific mediators, including uEPX/c, are increased.[27,28] However, we found that the presence or absence of atopic eczema did not influence the correlations between uEPX/c and the percentage eosinophils or ECP in induced sputum. We can not exclude that heterogeneity of study groups with respect to other atopic disorders than asthma could have affected the correlation between uEPX/c and other markers of eosinophilic airways inflammation.

At the onset of our study a circadian rhythm of uEPX/c had not been reported. Urine samples were not all obtained at the same time of the day. Since the start of our study it became evident that a circadian rhythm of uEPX/c with lowest levels at 7 pm and highest at 7 am in both asthmatic and healthy controls exists.[23,29-31] Hence, diurnal variability may have introduced scatter of uEPX, thus weakening a possible correlation.

Two previous studies reported significant positive correlations between uEPX/c and FE_{NO} in corticosteroid dependent childhood asthma.[11,29] We found no significant correlation between uEPX/c and FE_{NO} in a small subgroup of the study population. For FE_{NO}, no important circadian variation was found, employing the same measurement technique that we have used, but conflicting results have also been published.[27,32] A possible circadian rhythm might have affected FE_{NO} and weakened any cross-sectional relationship.

In conclusion, the present data show a weak inverse correlation between uEPX/c and FEV_1, and a borderline correlation between uEPX/c and PD_{20}methacholine. No significant correlation was found between uEPX/c and markers of eosinophilic airway inflammation including % eosinophils or ECP levels in induced sputum or FE_{NO}. The number of children performing Feno was small, therefore this correlation should be interpreted with caution. Our findings are not encouraging for uEPX/c as a complementary marker of airway inflammation in asthma. As to whether uEPX/c can be useful as a marker for monitoring asthma management in children is worth prospectively looking at.

REFERENCES

1 Busse W, Banks-Schlegel SP, Larsen GL. Childhood- versus adult-onset asthma. Am J Respir Crit Care Med 1995;151:1635-9.

2 Cai Y, Carty K, Henry RL, Gibson PG. Persistence of sputum eosinophilia in children with controlled asthma when compared with healthy children. Eur Respir J 1998;11:848-53.

3 Jatakanon A, Lim S, Kharitonov SA, et al. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. Thorax 1998;53:91-5.

4 Sont JK, Willems LN, Bel EH, et al. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med 1999;159:1043-51.

5 Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.

6 Gibson PG. Use of induced sputum to examine airway inflammation in childhood asthma. J Allergy Clin Immunol 1998;102:S100-1.

7 Piacentini GL, Bodini A, Costella S, et al. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. Eur Respir J 1999;13:1386-90.

8 Gibson PG, Henry RL, Thomas P. Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate. Eur Respir J 2000;16:1008-15.

9 Reimert CM, Minuva U, Kharazmi A, et al. Eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN). Detection by enzyme-linked immunosorbent assay and purification from normal human urine. J Immunol Methods 1991;141:97-104.

10 Cottin V, Deviller P, Tardy F, et al. Urinary eosinophil-derived neurotoxin/protein X: a simple method for assessing eosinophil degranulation in vivo. J Allergy Clin Immunol 1998;101:116-23.

11 Mattes J, Storm van's Gravesande K, Reining U, et al. NO in exhaled air is correlated with markers of eosinophilic airway inflammation in corticosteroid-dependent childhood asthma. Eur Respir J 1999; 13:1391-5.

12 Severien C, Artlich A, Jonas S, et al. Urinary excretion of leukotriene E4 and eosinophil protein X in children with atopic asthma. Eur Respir J 2000;16:588-92.

13 Hoekstra MO, Hovenga H, Gerritsen J, et al. Eosinophils and eosinophil-derived proteins in children with moderate asthma. Eur Respir J 1996;9:2231-5.

14 Kristjansson S, Strannegard IL, Strannegard O, et al. Urinary eosinophil protein X in children with atopic asthma: a useful marker of antiinflammatory treatment. J Allergy Clin Immunol 1996;97:1179-87.

15 Baraldi E, de Jongste JC. Measurement of exhaled nitric oxide in children, 2001. Eur Respir J 2002; 20:223-37.

16 Quanjer PH, Tammeling GJ, Cotes JE, et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society [see comments]. Eur Respir J Suppl 1993;16:5-40.

17 Zapletal A, Samanek M, Paul T. Lung function in children and adolescents. Methods and reference values. Progress in respiration research. Basel;Karger,1987;22:114-218.

18 Birnie D, Thoe Schwartzenberg GW, Hop WC, et al. Does the outcome of the tidal breathing and dosimeter methods of assessing bronchial responsiveness in children with asthma depend on age? Thorax 1990;45:199-202.

19 Efthimiadis A, Spanevello A, Hamid Q, et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. Eur Respir J Suppl 2002;37:19s-23s.

20 in 't Veen JC, de Gouw HW, Smits HH, et al. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. Eur Respir J 1996;9:2441-7.

21 Lugosi E, Halmerbauer G, Frischer T, et al. Urinary eosinophil protein X in relation to disease activity in childhood asthma. Allergy 1997;52:584-8.

22 Oosaki R, Mizushima Y, Kawasaki A, et al. Correlation among urinary eosinophil protein X, leukotriene E4, and 11-dehydrothromboxane B2 in patients with spontaneous asthmatic attack. Clin Exp Allergy 1998;28:1138-44.

23 Labbe A, Aublet-Cuvelier B, Jouaville L, Beaugeon G, et al. Prospective longitudinal study of urinary eosinophil protein X in children with asthma and chronic cough. Pediatr Pulmonol 2001;31:354-62.

24 Piacentini GL, Peterson C, Peroni DG, et al. Allergen avoidance at high altitude and urinary eosinophil protein X. J Allergy Clin Immunol 1999;104:243-4.

25 Hoekstra MO, Grol MH, Bouman K, et al. Fluticasone propionate in children with moderate asthma. Am J Respir Crit Care Med 1996;154:1039-44.

26 Koller DY, Halmerbauer G, Frischer T, et al. Assessment of eosinophil granule proteins in various body fluids: is there a relation to clinical variables in childhood asthma? Clin Exp Allergy 1999;29:786-93.

27 Oymar K, Bjerknes R. Urinary eosinophil protein X in children with atopic dermatitis: relation to atopy and disease activity. Allergy 2000;55:964-8.

28 Pucci N, Lombardi E, Novembre E, et al. Urinary eosinophil protein X and serum eosinophil cationic protein in infants and young children with atopic dermatitis: correlation with disease activity. J Allergy Clin Immunol 2000;105:353-7.

29 Mattes J, Storm van 's Gravesande K, Moeller C, et al. Circadian variation of exhaled nitric oxide and urinary eosinophil protein X in asthmatic and healthy children. Pediatr Res 2002;51:190-4.

30 Storm van 's Gravesande K, Mattes J, Gruntjens T, et al. Circadian variation of urinary eosinophil protein X in asthmatic and healthy children. Clin Exp Allergy 1999;29:1497-501.

31 Schosser M, Hoeppe P, Radon K, et al. Circadian rhythm of U-EPX. Allergy 2001;56:792-3.

32 Kharitonov SA, Gonio F, Kelly C, et al. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. Eur Respir J 2003;21:433-8.

Chapter 6

Urinary Eosinophil Protein X in childhood asthma: relation with changes in disease control and eosinophilic airway inflammation

Marianne Nuijsink, Wim C.J. Hop, Peter J. Sterk,
Eric J. Duiverman and Johan C. de Jongste

Mediators of Inflammation 2012

6

6 Urinary Eosinophil Protein X in childhood asthma: relation with changes in disease control and eosinophilic airway inflammation

ABSTRACT

The aim of this study was to assess cross-sectional and longitudinal correlations between uEPX and other markers of asthma control and eosinophilic airway inflammation.

Methods

We measured uEPX at baseline, after 1 yr and after 2 yrs in 205 atopic asthmatic children using inhaled fluticasone. At the same time points, we assessed symptom scores (2 weeks diary card), lung function (forced expiratory volume in one second (FEV_1)) and airway hyperresponsiveness (AHR) and percentage eosinophils in induced sputum(%eos)

Results

We found negative correlations between uEPX and FEV_1 at baseline (r= -0.18, p=0.01), after 1 year(r= -0.25, p<0.01) and after 2 years(r= -0.21, p=0.02). Within-patient changes of uEPX showed a negative association with FEV_1 changes (at 1 yr r= -0.24, p=0.01; at 2 yrs: r= -0.21, p=0.03). Within-patient changes from baseline of uEPX correlated with changes in % eos. No relations were found between uEPX and symptoms.

Conclusion

In this population of children with atopic asthma, uEPX correlated with FEV_1 and %eos, and within-subjects changes in uEPX correlated with changes in FEV_1 and %eos. As the associations were weak, and the scatter of uEPX wide, it seems unlikely that uEPX will be useful as a biomarker for monitoring asthma control in the individual child.

INTRODUCTION

Eosinophilic protein X is produced by activated eosinophils, and can be measured in blood, stools and urine (uEPX).[1,2] Elevated uEPX has been reported in asthmatics with or without allergy, with great overlap between healthy controls and asthmatics.[3-6] A relation with asthma symptoms and lung function has been shown, and uEPX increases significantly during asthma exacerbations.[7-9] A reduction in uEPX has been reported after starting inhaled corticosteroids.[11] Therefore, it has been suggested that measuring urinary EPX may be useful for monitoring the effect of ICS in asthma.[3,10,11]

Since measuring uEPX is completely non-invasive and independent of patient's co-operation it has the potential to be a feasible tool in asthma management in young children. Several studies have demonstrated cross-sectional correlations between uEPX and conventional clinical markers of asthma control and airway inflammation.[3,4,12] Previously we reported these cross-sectional data measured at baseline in part of the present study population.[13] Data on longitudinal changes in uEPX within subjects compared to changes in markers of asthma control or airway inflammation were examined in only one study in 14 children.[3]

We analysed cross-sectional and longitudinal uEPX data from a long-term follow-up study in children with atopic asthma.[14] We hypothesised that changes in uEPX would be related to changes eosinophilic airway inflammation, and could provide information that is additional to symptoms and lung function.

METHODS

Subjects

Asthmatic children took part in a randomised controlled multi-centre trial (CATO: Children Asthma Therapy Optimal), reported in detail previously.[14] Briefly, 288 children with a documented clinical history of moderately severe asthma were recruited from 15 paediatric clinics including 7 university hospitals in the Netherlands. During a run-in phase, all patients were treated with 100 or 250 µg fluticasone bid depending on their equivalent treatment before the study. Children were selected on the basis of symptoms and/or AHR, and 210 children were randomised to a reference strategy (adjustment of treatment on symptom score) or an AHR- strategy (treatment adjusted on the basis of AHR and symptom score). Every 3 months treatment was adjusted, during a 2-year follow-up. All subjects received a dry powder inhaler containing fluticasone 100 or

200 µg per day or fluticasone/salmeterol 200/100, 500/100 or 1000/100 µg per day (Flixotide Diskus® or Seretide Diskus®, GlaxoSmithKline, UK). For the present study, 'baseline' is defined as the moment of randomisation for the CATO study.

Symptom scores

Patients filled in a diary card daily during 2 weeks before each 3-months visit. Cough, shortness of breath and wheezing during night and day were recorded on a 4 point scale (0=no symptoms to 3=severe symptoms interfering with activity or sleep).[15] The percentage of symptom-free days defined as score 0 for cough, wheeze and shortness of breath was calculated.

AHR and spirometry

were measured during each clinic visit.[16] Study medication was stopped 36 hours before lung function and AHR testing. AHR was tested by methacholine challenge using a dosimeter method.[17] The provocation dose causing a 20% fall in FEV_1 from baseline (PD_{20}) was calculated from a log dose-response plot by linear interpolation.

Sputum induction and processing

Sputum induction was attempted in 8 of the 15 participating centres. Sputum was induced according to a standardised method by inhaling an aerosol of hypertonic sodium chloride 4.5% w/v.[18,19] Differential cell counts of cytospins were performed and samples containing more than 80% squamous cells were excluded from the analysis.[18]

Urinary Eosinophil Protein X

Spot samples of urine were collected at randomisation (= baseline), after a treatment period of 1 year, and after 2 years (the end of the study), and immediately stored at -20° C. EPX was determined using a commercial enzyme-linked immunosorbent assay (ELISA) for human EPX in 50-fold diluted samples (Medical and Biological Laboratories, Nakaku Nagoya, Japan). The essay's sensitivity was 0.62 ng/ml. Urinary creatinine levels were measured by the alkaline picrate method (Roche, Mannheim, Germany). Urinary EPX concentrations were expressed as µg per mmol creatinine.

Statistical analysis

Repeated measurements Anova was used to evaluate associations between the various markers and uEPX levels. The same method was used to assess changes from baseline. With this method (SAS PROC MIXED) the information about the associations at the different study time points can be combined and the method allows for uEPX not being available at all three time points for each patient. In these analyses uEPX values, %eosinophils in sputum and fluticasone doses were transformed logarithmically in order to get approximate normal distributions and to reduce the effect of outlying observations, and consequently results for these parameters are expressed as geometric means. Percentage eosinophil values equal to 0 were replaced by 0.1% in order to allow the logarithmic transformation. In a substantial part of the study population, especially at time points T1 and T2, PD_{20} was higher than the highest dose methacholine used (1570 µg). For that reason PD_{20} data were arbitrarily dichotomized in non-AHR (PD_{20}>300 µg) and AHR (PD_{20}<300 µg). Correlation coefficients calculated at the different time points are Spearman's rank correlations. P=0.05 (two-sided) was considered the limit of significance.

RESULTS

Urinary EPX was measured at least once in 205 children. Patient characteristics at baseline are given in table 1.

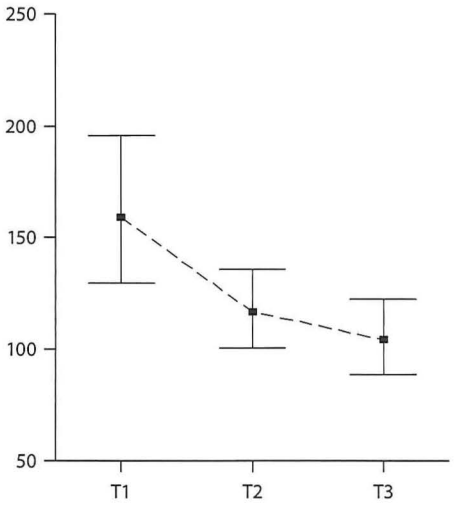
Table 1 *- Characteristics of the 205 children at baseline. FEV_1, forced expiratory volume in 1 second; PD_{20}methacholine, provocative dose of methacholine causing FEV_1 fall 20% from baseline.*
** 12 children had not reached the 20% fall at the highest dose of 1570 µg*
*** 79 patients had a total of 127 % eos measurements combined with an uEPX measurement, and in 49 of these a baseline measurement was available*

Age (y)	10.4 [6.4-16.8]
Gender (m/f)	118 / 87
Daily dose fluticasone 200 µg/ 500 µg	116 / 89
uEPX (µg/mmol creatinine) (n=180)	184 (2-3114)
Symptom-free days (%)	50 (0-100)
FEV_1 (%pred)	97 (56-136)
PD_{20}methacholine (µg)	72 (1->1570.0*)
Sputum eosinophils (%) (n=49)**	1 (0-72)

Values are median (range)

In total 461 uEPX samples were analysed during the study. During the 2 years treatment period, the geometric mean uEPX significantly decreased from 159 µg/mmol to 104 µg/mmol (p<0.001) (figure 1).

Figure 1 *- uEPX levels over time. Geometric mean uEPX (per mmol creatinine) levels with 95 percent confidence intervals by measurement visit (Anova estimates). T0: baseline (n=180); T1: after a treatment period of 1 year (n=148); T2: after a treatment period of 2 years (n=133). T1 versus T0: p=0.01; T2 versus T1: p=0.18; T2 versus T0: p<0.001.*



Anova showed a significant treatment effect after 1 year: In the AHR strategy group uEPX had decreased significantly more than in the reference strategy group, with a 29% lower geometric mean EPX level at that time point (p=0.03). No treatment effect was found at year 2 (p=0.26). Further analysis showed that at 1 year there was a significant fluticasone dose effect, and this effect was not found at baseline or at year 2. At 1 year a two-fold higher dose was associated with a 25% lower uEPX level (95% CI: 15%-34%; p<0.001). Children treated according to the AHR strategy had received significantly higher doses of fluticasone at 1 year (geometric means 580 vs. 412 ug/day; Mann-Whitney test: p=0.01). After adjustment for the higher dose using Anova, no significant treatment effect remained.

Table 2 *- Correlations between uEPX and clinical markers of asthma*
T0: at baseline; T1: after a treatment period of 1 year; T2: after a treatment period of 2 years; FEV_1, forced expiratory volume in 1 second. Numbers evaluated for sputum eosinophils at T0, T1 and T2 are 49, 45 and 33, respectively. Data shown represent Spearman rank correlation coefficients.

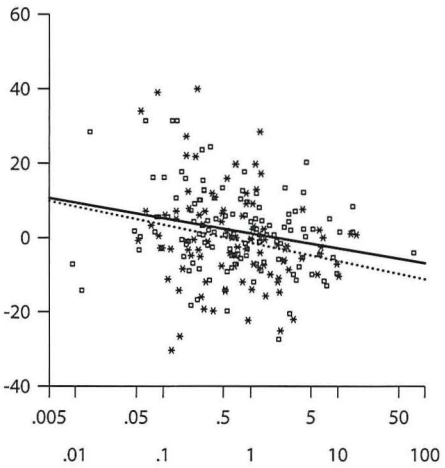
		r	p-value
Symptom-free days (%)	T0	-0.04	0.96
	T1	0.03	0.68
	T2	0.09	0.34
FEV_1 (%pred)	T0	-0.18	0.01
	T1	-0.25	<0.01
	T2	-0.22	0.02
Sputum eosinophils (%)	T0	0.19	0.19
	T1	0.19	0.20
	T2	0.51	<0.01
PD_{20}methacholine (µg)	T0	-0.14	0.08
	T1	-0.20	0.02
	T2	-0.14	0.14

Table 3 *- Correlations between the changes from baseline in uEPX and changes in clinical markers of asthma control. T0: at baseline; T1: after a treatment period of 1 year; T2: after a treatment period of 2 years. FEV_1, forced expiratory volume in 1 second. The individual changes from baseline of uEPX and % eos are expressed as ratios. For FEV_1 and symptom free days the changes are expressed as the absolute differences of the measured percentages. Numbers evaluable for the change from baseline of %eos at T1 and T2 are 23 and 17, respectively. Data shown are Spearman rank correlations.*

		r	p-value
Symptom-free days (%)	T1	-0.06	0.53
	T2	0.10	0.32
FEV_1 (%pred)	T1	-0.24	0.01
	T2	-0.21	0.03
Sputum eosinophils (%)	T1	0.10	0.64
	T2	0.40	0.11

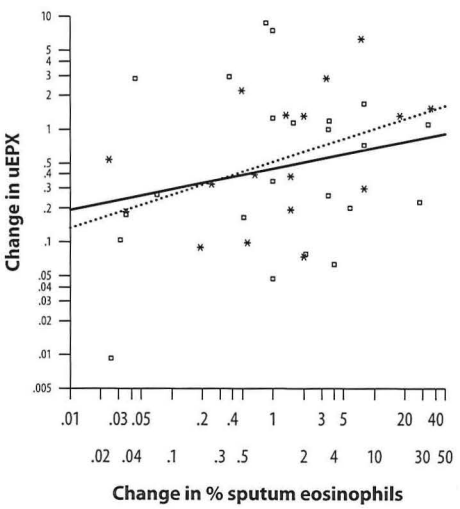
No correlations were found between uEPX and the percentage symptom free days or changes in uEPX and changes in percentage symptom-free days. Anova also did not show significant associations. Cross-sectional analysis showed weak negative correlations of the levels of uEPX and FEV_1 (baseline: r= -0.18, p=0.02; at 1 year: r= -0.25, p=0.002; at 2 years: r= -0.21, p=0.016, respectively). Anova showed that the significant associations did not differ between the three time points and adjusting for ICS dose did not change this. Also the changes from baseline of uEPX and FEV_1 were significantly related with each other (at 1 year r= -0.24, p=0.01 and at 2 years; r= -0.21, p=0.03) (figure 2). As compared to baseline, a ten-fold increase in uEPX was associated with a decrease of FEV_1 (%pred) of 2.8 percentage points (95%CI: 0.3 to 5.3; p=0.026) and this decrease did not significantly differ between the two time points (p=0.65). A similar result was found when changes from the previous assessment were evaluated.

Figure 2 *- Changes from baseline in lung function versus changes in uEPX. Squares and the solid regression line pertain to changes between baseline and 1 year follow-up, asterisks and the dashed line represent changes between baseline and 2 yrs. Changes of uEPX are shown as ratios; changes of FEV_1 are shown as absolute differences of percentage predicted values.*



At all visits uEPX levels were higher in patients with AHR (PD_{20}methacholine <300 µg methacholine) than in patients without AHR. At 1 year this difference was statistically significant, with 13% higher uEPX in patients with AHR (p=0.006) (p=0.08, p=0.10 respectively at baseline and after 2 years treatment). This effect remained after adjustment for dose. With regard to percentage eosinophils in sputum a significant correlation with uEPX was found at 2 years (r=0.51, p<0.001, n=33). Anova showed a significant overall association between uEPX and percentage sputum eosinophils. A two-fold higher value of sputum eosinophils was associated with a 14% increased value of uEPX (95%CI: 6% - 22%; p<0.001), and this relation did not significantly differ between the 3 time points (p=0.11), nor was the relation affected by ICS dose. Although changes from baseline of uEPX and percentage sputum eosinophils did not significantly correlate with each other at the separate time points, a significant relation was found combining both time points using Anova. A within patient doubling of sputum eosinophils was associated with a mean increase of uEPX of 16% (95% CI: 0.1%-36%; p=0.049), and the ICS dose did not affect this relation. The individual variations however are large (figure 3).

Figure 3 *- Changes from baseline in uEPX versus changes in sputum eosinophils. Squares and the solid regression line pertain to the changes between baseline and 1 year (n=23), asterisks and the dashed line represent changes between baseline and 2 yrs (n=17). Changes of uEPX and percentage sputum eosinophils are both shown as ratios.*



DISCUSSION

We prospectively assessed changes in uEPX in relation to changes in asthma control within-subjects over an observation period of 2 years. We found in atopic asthmatic children that changes in uEPX measured after 1 and 2 years related significantly with changes in FEV_1 and % sputum eosinophils, but not with changes in symptoms. Changes in uEPX correlated with changes in ICS dose.

Conflicting data have been reported about cross-sectional relationships between uEPX and FEV_1. Previously we described the results of cross-sectional analysis at baseline in the present study population.[13] We found a weak inverse correlation between uEPX and FEV_1. With the present study we confirmed the relation between uEPX and FEV_1 at different time points. Moreover we showed that changes in uEPX and changes FEV_1 were related. This is in line with the significant correlation between these parameters found by Lugosi in 14 asthmatic children.[3] However the interval of their measurements was much shorter (1-2 months). It has been shown that correlations between eosinophilic airway inflammation and lung function are weak and may only reach significance in large populations.[4,6,20,21] Clearly FEV_1 is dependent on many more factors than eosinophilic inflammation alone.

The correlation between uEPX and sputum eosinophils was weak, and this is in line with the relations between sputum ECP, sputum eosinophils and uEPX described by Mattes.[12] Weak correlations seem to be the rule when different markers of eosinophilic inflammation are compared.[22] Moreover, uEPX is secreted by activated eosinophils, and it is therefore possible that uEPX provides information about eosinophil activation rather than numbers.

We found no relation between uEPX and symptoms. A significant relation between uEPX and symptoms has been described during acute asthma exacerbations, spontaneously or provoked by ICS withdrawal.[23] Also, significantly higher uEPX levels were found in symptomatic asthmatic children than in asymptomatic children.[1,3,4] However, a 6 months follow up study in 14 children with mild asthma found no significant association was found.[9] So the severity of symptoms should be taken into account when correlating uEPX and symptoms. In our study population the symptom scores were low and this may explain the apparent discrepancy with these previous studies. It could well be that relations between uEPX and parameters of asthma control could have been influenced by other factors as well, including seasonal variations, severity of allergy and asthma and atopic dermatitis.[24,25] The use of varying doses of ICS is another possibility, for which we have corrected.

What are the clinical implications of our findings? As uEPX does not correlate with symptoms, but does correlate with sputum eosinophils, we could argue that uEPX might provide additional information on activity of eosinophilic airway inflammation. However, the correlations were weak and the scatter of individual uEPX values wide. Therefore it seems unlikely that uEPX will be useful as a biomarker for monitoring asthma in the individual child.

REFERENCES

1. Koller DY, Halmerbauer G, Frischer T, Roithner B. Assessment of eosinophil granule proteins in various body fluids: is there a relation to clinical variables in childhood asthma? Clin Exp Allergy 1999: 29: 786-93.
2. Majamaa H, Laine S, Miettinen A. Eosinophil protein X and eosinophil cationic protein as indicators of intestinal inflammation in infants with atopic eczema and food allergy. Clin Exp Allergy 1999: 29: 1502-6.
3. Lugosi E, Halmerbauer G, Frischer T, Koller DY. Urinary eosinophil protein X in relation to disease activity in childhood asthma. Allergy 1997: 52: 584-8.
4. Tauber E, Halmerbauer G, Frischer T et al. Urinary eosinophil protein X in children: the relationship to asthma and atopy and normal values. Allergy 2000: 55: 647-52.
5. Hoekstra MO, Hovenga H, Gerritsen J, Kauffman HF. Eosinophils and eosinophil-derived proteins in children with moderate asthma. Eur Respir J 1996: 9: 2231-5.
6. Severien C, Artlich A, Jonas S, Becher G. Urinary excretion of leukotriene E4 and eosinophil protein X in children with atopic asthma. Eur Respir J 2000: 16: 588-92.
7. Oosaki R, Mizushima Y, Kawasaki A, Mita H, Akiyama K, Kobayashi M. Correlation among urinary eosinophil protein X, leukotriene E4, and 11-dehydrothromboxane B2 in patients with spontaneous asthmatic attack. Clin Exp Allergy 1998: 28: 1138-44.
8. Khalil Kalaajieh W, Hoilat R. Asthma attack severity and urinary concentration of eosinophil X protein in children. Allergol Immunopathol 2002: 30: 225-31.
9. Wojnarowski C, Roithner B, Koller DY et al. Lack of relationship between eosinophil cationic protein and eosinophil protein X in nasal lavage and urine and the severity of childhood asthma in a 6-month follow-up study. Clin Exp Allergy 1999: 29: 926-32.
10. Kristjansson S, Strannegard IL, Strannegard O, Peterson C, Enander I, Wennergren G. Urinary eosinophil protein X in children with atopic asthma: a useful marker of antiinflammatory treatment. J Allergy Clin Immunol 1996: 97: 1179-87.
11. Labbe A, Aublet-Cuvelier B, Jouaville L et al. Prospective longitudinal study of urinary eosinophil protein X in children with asthma and chronic cough. Pediatr Pulmonol 2001: 31: 354-62.
12. Mattes J, Storm van's Gravesande K, Reining U et al. NO in exhaled air is correlated with markers of eosinophilic airway inflammation in corticosteroid-dependent childhood asthma. Eur Respir J 1999: 13: 1391-5.
13. Nuijsink M, Hop WC, Sterk PJ, Duiverman EJ, Hiemstra PS, de Jongste JC; Urinary eosinophil protein X in children with atopic asthma. Mediators Inflammation 2007: 2007: 49240.

14. Nuijsink M, Hop WC, Sterk PJ, Duiverman EJ, de Jongste JC. Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial. Eur Respir J 2007: 30: 457-66.
15. Verberne AA, Frost C, Roorda RJ, van der Laag H, Kerrebijn KF. One year treatment with salmeterol compared with beclomethasone in children with asthma. The Dutch Paediatric Asthma Study Group. Am J Respir Crit Care Med 1997:156: 688-95.
16. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993: 16: 5-40.
17. Birnie D, 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: 45: 199-202.
18. Efthimiadis A, Spanevello A, Hamid Q et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. Eur Respir J 2002: 37: 19s-23s.
19. in 't Veen JC, de Gouw HW, Smits HH et al. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. Eur Respir J 1996: 9: 2441-7.
20. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000: 161: 9-16.
21. van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med 2001: 164: 2107-13.
22. Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. Clin Exp Allergy 2005: 35: 1175-9.
23. Lonnkvist K, Hellman C, Lundahl J, Hallden G, Hedlin G. Eosinophil markers in blood, serum, and urine for monitoring the clinical course in childhood asthma: impact of budesonide treatment and withdrawal. J Allergy Clin Immunol 2001: 107: 812-7.
24. Oymar K, Bjerknes R. Urinary eosinophil protein X in children with atopic dermatitis: relation to atopy and disease activity. Allergy 2000: 55: 964-8.
25. Pucci N, Lombardi E, Novembre E et al. Urinary eosinophil protein X and serum eosinophil cationic protein in infants and young children with atopic dermatitis: correlation with disease activity. J Allergy Clin Immunol 2000: 105: 353-7.

Chapter 7

General discussion

7 General discussion

Pediatric asthma remains a health care problem on a global scale, despite the availability of evidence based treatment plans.[1] Asthma can not be cured, and is often a lifelong problem. This emphasizes the need for optimal treatment and monitoring of pediatric asthma. Effective medication is available for children with asthma: inhaled corticosteroids have proven to be very effective on a group level. However, asthma is a heterogeneous disease in which different factors have a role in pathogenesis, and therefore it seems a priori unlikely that a single treatment modality would be suitable for all patients. As asthma control is often insufficient despite the availability of proper asthma medication, and because asthma is a heterogeneous disease, the hypothesis has been put forward that asthma treatment should be more phenotype-driven.

In this thesis we have shown benefit of such a phenotype-driven asthma treatment strategy. We developed a treatment algorithm that was based on the level of airway hyperresponsiveness (AHR), and showed that this specifically benefited a large subgroup of children characterized by low symptom scores despite AHR. This underlines the need for better phenotyping of patients, in order to treat them according to individualized management plans. We also concluded that, after two years of AHR-driven treatment, the benefit of this strategy was lost within the next five years.

One of the explanations for our findings could be that patients with more severe AHR do not perceive bronchoconstriction correctly, and would therefore be at risk for undertreatment of their asthma. This was supported by our finding of a negative correlation between AHR and perception of bronchoconstriction. We would like to make a case for routinely assessing patients' perception of bronchoconstriction and taking this in consideration in the individual management plan.

Another aspect of phenotype-specific treatment is that the presence and nature of airway inflammation should be taken into account. In children, many of the markers studied in adults are problematic, including markers in induced sputum or serum, and lung function. For that reason we investigated whether urinary eosinophilic protein X (uEPX), which derives from activated eosinophils and might reflect eosinophilic inflammation, would be a useful biomarker. We found that this marker correlates with some aspects of disease activity in a large group of atopic asthmatic children. Unfortunately, the wide scatter of individual uEPX values and limited agreement with conventional measures of asthma severity and control are not very promising for further development of uEPX as biomarker for individual children.

How appropriate is today's childhood asthma treatment?

Based on recent insights, asthma guidelines propose that treatment should aim at achieving and maintaining asthma control. In the global initiative for asthma (GINA) guidelines, the assessment of asthma control is based on daytime symptoms, nocturnal symptoms, limitations of activity, need for rescue treatment, lung function and asthma exacerbations.[2] Level of control is divided in: controlled, partly controlled and uncontrolled (see table 1), with arbitrary cutoffs.

Table 1 *- Levels of asthma control*

	Controlled	Partly controlled	Uncontrolled
Description	All of the below measures	Any measure present in any week	≥ 3 features of partly controlled asthma present in any week
Daytime symptoms	None**	>2 per week	≥ 3 features of partly controlled asthma present in any week
Limitations of activity	None	Any	
Nocturnal symptoms	None	Any	
Need for rescue treatment	None**	> 2 per week	
Lung function*	Normal	< 80% predicted or personal best	
Exacerbations	None	≥ 1 per year	1 in any week

**: forced expiratory volume in 1 s or peak expiratory flow; **: < 3 per week*

GINA states that, although there is no cure for asthma, appropriate management most often results in the achievement of control. This is in line with studies on the ability to achieve and maintain asthma control with intensive, accessible, guidelines-defined care with close follow up.[3,4] However, in daily life, a substantial proportion of children asthma is not optimally controlled and the burden of childhood asthma is still substantial, with high frequencies of sleep disturbances, emergency visits, school absence and limitations of physical activity due to asthma.[1,5-7] In a large population of Dutch school children undiagnosed asthma was more frequent than correctly diagnosed asthma.[8] Asthma is also seriously under-diagnosed among adolescents, with as much as one third of patients not diagnosed.[9] This obviously increases the risk of undertreatment. But, also in children with doctor's diagnosed asthma, considerable undertreatment as well as overtreatment has been shown.[10,11] Overall, parents, children and adolescents overestimate asthma control and underestimate asthma severity. This is

an important obstacle to successful asthma treatment in children, and may stand in the way of reaching the goals of good asthma control as defined by GINA.[7] Clearly, an appropriate diagnosis with characterization of relevant aspects of the phenotype, followed by personalized treatment plans, would be a major step forward.

What have we learned from phenotype driven asthma treatment?

According to de definition of asthma, treatment plans could be tuned on the presence of symptoms, airways obstruction and reversibility, AHR and the presence and nature of airway inflammation; in addition, the dynamics of these aspects could be taken into account, including fluctuations in lung function or inflammation.[12,13] In daily practice, such an approach is apparently not feasible, and treatment guidelines are based on symptoms, with or without measures of lung function. This may well explain the poor overall treatment effects, as the response to therapy may be variable for the different phenotypes. Indeed, by using cluster analysis, it has been shown that identification of asthma phenotypes that exhibit differences in clinical response to treatment algorithms is feasible.[14]

Treatment based on symptoms

Traditionally, asthma treatments have been individualized using symptoms as the main read-out. Bad perception of bronchoconstriction is a major pitfall in this way of asthma management. In a Dutch study, more than one third of the asthmatic children had poor perception, about one in five over-reported symptoms and in one in four there was a complete dissociation between symptoms and lung function.[15] We showed in this thesis that the level of AHR itself is a risk factor for poor perception of bronchoconstriction. When relying on symptoms, physicians should be aware of the great variability of perception of bronchoconstriction, between patients and within a patient over time. So, although symptoms will probably remain of great importance in asthma management plans, they are only part of the asthma syndrome, and certainly the most subjective one. To overcome this, specific short questionnaires have been developed and validated that assess symptoms in a standardized way. However, there is still a lack of data on the effectiveness of using such questionnaires in asthma management.

Treatment based on lung function and airway hyperresponsiveness

Lung function tests have long been used as a means to objectively support a clinical diagnosis of asthma. Repeated peak expiratory flow (PEF) measurements and spirometry to identify variable airflow obstruction and reversibility to bronchodilator have been extensively studied. In the 1980s test for AHR were increasingly used as well. Lung function and AHR characterize the functional impact of the underlying airways pathology, but show limited correlation with the airway pathology itself. Disappointingly, there is no evidence that regular monitoring of lung function improves asthma control in children. Changes in PEF poorly reflect changes in asthma activity.[16] Peak expiratory flow records are unreliable, and self-management plans that take PEF into account are no more effective than plans based on symptom monitoring alone.[17,18] Things are different for AHR, which in a sense was the first biomarker explored as a tool in an individualized asthma management plan to guide inhaled corticosteroid treatment. Benefits of AHR-driven asthma management have been shown in adults with asthma, and resulted in improved lung function and a significant reduction in the number of asthma exacerbations. In this thesis we investigated AHR as phenotypic characteristic to improve treatment in asthmatic children. We showed preservation of lung function as a result of the AHR strategy with the greatest benefit in children with low symptom scores despite AHR. This underlined that a strategy based on symptoms alone was insufficient for individuals in this subgroup, and that phenotyping is important for the success of treatment.

Treatment based on airway inflammation

Although the eosinophilic granulocyte is an important player in the field of allergy and asthma, being allergic is not identical to having eosinophilic inflammation in the airways. Adult patients with intrinsic (non atopic) and extrinsic (atopic) asthma have similar airway pathology.[19] Studies using endobronchial biopsies to assess airway pathology in children with multitrigger wheeze showed no structural or functional differences between atopic and non atopic children.[20]

Eosinophils in sputum and exhaled nitric oxide (FeNO) have been shown reliable biomarkers that both reflect eosinophilic airway inflammation. Sputum eosinophils can predict the response to ICS, and are predictive for the risk of an asthma exacerbation. Asthma treatment based on sputum eosinophils is effective in reducing the risk of asthma exacerbations in adults, without overall increasing in mean dose inhaled corticosteroids (ICS). As could be expected, treatment strategies based on findings in sputum were of particular benefit in patients with eosinophilic airway inflammation and few symptoms. Up titrating of ICS in this group in order to control sputum eosinophilia resulted in a markedly reduced exacerbation rate. Conversely, ICS could be safely reduced patients with severe symptoms but no eosinophilic airway inflammation. In view of these findings in adults, incorporating the measurement of sputum eosinophils into the treatment algorithm in children with severe asthma seemed a potentially attractive strategy to reduce the exacerbation rate. Unfortunately, findings in children were different from those reported for preceding studies in adult asthma patients, and not as successful.[21] The main limitations for using sputum samples in children with asthma are the high technical demands and cost of the sputum induction and analysis. Moreover, a substantial proportion of children with mild-moderate severe asthma fail to produce adequate sputum samples on repeated occasions, which is a prerequisite for use as biomarker on which to base treatment. At this time, with present equipment for sputum induction and processing, these technique are only suitable for research. The most easily measured and readily accessible biomarker of eosinophilic airway inflammation is FeNO. Measurements of FeNO prior to beginning treatment may be useful in predicting response to ICS therapy in children and FeNO can predict the risk of asthma exacerbations. Hence, FeNO seemed a promising marker on which the dose of inhaled corticosteroids in asthma could be titrated, thus tailoring the treatment to those patients who actually have chronic eosinophilic airway inflammation. A limited number of studies explored the effect of FeNO-driven asthma treatment. The results were inconsistent, with marked benefits in certain populations and no benefits in others. Two Cochrane reviews concluded that there was insufficient evidence that tailoring of asthma treatment based on FeNO monitoring improved asthma outcome in children. However, these reviews were compromised by inconsistency between studies with respect to differences in populations, treatment algorithms, and definitions of outcomes.

Are inflammatory phenotypes in asthma the same in adults and in children?

The character and significance of airway inflammation in adults and children with asthma seem to be different. In most children with stable asthma, airway eosinophils and neutrophils are in the normal range. In contrast, eosinophilic inflammation is more likely in adult asthmatics.[22] The presence of eosinophilic inflammation predicts more severe persistent asthma with impaired lung function and increased AHR in children. In adult asthmatics this association is not clear. Adults and children with asthma may also differ in their response to ICS. In adults it is clear that non-eosinophilic asthma is associated with an attenuated response to corticosteroids.[23,24] This has not been shown for pediatric asthma. In children, improvements in lung function after systemic corticosteroid was independent of the presence of eosinophils in sputum before treatment.[25] One should realize that such studies pertain to specific, highly selected patients, and may not apply to daily practice. The above findings indicate that clinical features in children and adults with asthma do not necessarily mean the same thing in terms of pathophysiology and treatment response. They highlight the need for specific, pediatric studies when defining the role of phenotype-specific treatment algorithms

Are asthma phenotypes stable?

Given that asthma is a variable disease, one can assume that asthma phenotypes are not stable over time. Most studies of inflammatory phenotypes in asthma confine their analyses to cross-sectional data. In children with severe asthma, phenotypic variability was seen with 63% of children demonstrating a change in inflammatory phenotype on repeated assessment within a year. In contrast, asthma in adults is associated with greater phenotypic stability.[26] When we performed a follow-up study of the children that we treated initially for 2 years according to our AHR- and symptom based strategies, we noticed that many had changed phenotype within a five year interval. In particular, a substantial number of the children had much less symptoms and no AHR at follow-up. However, children who originally had symptoms but no AHR remained without AHR. Our findings do not allow for conclusions about the time course of phenotypic changes, as only a single follow-up visit was planned.
Knowledge about the variability of specific phenotypes is desirable, as this will determine the preferred frequency of monitoring. Repeated re-assessment of phenotype is clearly important, and might affect treatment plans. As proposed recently by Anderson, perhaps we should now target our energy to define endotypes, stable subgroups defined by unique and specific genetic or molecular

characteristics, rather than phenotypes defined by biomarkers of disease activity, as these may lead to confusion and uncertainty as they change with time.[27] To demonstrate that such endotypes are indeed stable, and contribute to personalized medicine, will be a major challenge for the future.

There is a large number of potential biomarkers in asthma, including blood eosinophils, sputum eosinophilic cationic protein, and literally hundreds of components in exhaled breath condensate, that may be used in asthma monitoring. Evidence of a beneficial role in asthma management of any of these is lacking.

Do we need phenotyping, or should we focus on other aspects of asthma treatment?

In asthma management the present focus is on symptom-based levels of control.[1] At the moment there is no firm indication that monitoring asthma based on repetitive lung function measurement or markers of airway inflammation is superior to monitoring based on symptoms only. Asthmatic children commonly overestimate control and underestimate asthma severity. It is a great challenge for the physician to treat such children successfully. It would be naïve to think that poorly controlled asthma will improve by prescribing more drugs, as it has been shown that most children with poor asthma control are non-adherent to treatment.[28,29] Non-adherence is higher in subjects with much symptoms, therefore non-adherence should always be suspected in those with poorly controlled asthma.[30,31]

Studies using electronic monitoring devices to evaluate adherence with asthma medication reported adherence rates of 50-80%.[32,33] These figures are likely to be biased and higher than adherence within the general population.[34] Adherence as reported by parents or patients differs from objectively assessed adherence, and healthcare professionals are unable to estimate adherence, independent of the experience of the physician.[33,35] Children and parents significantly over reported their adherence, with reported adherence being outside the +/- 25% accuracy range for as much as half of the population.[36] So self report is inadequate to evaluate adherence. Assessing adherence might be used as a strategy to motivate patients to take medication, and for this purpose objective measures are desirable. Risk factors for non-adherence are non-supportive or overly anxious or controlling parents, children with behavioral difficulties, and families with lack of organized routine.[37-39] Adherence may be better if doctors communicate in a collaborative rather than authoritarian way.[40] People make decisions

unconsciously, instinctively, rather than mentally balancing pros and cons, and are strongly influenced by emotions and prejudices.[41] Thus, it is important not only to educate patients and parents, but to discover and address their specific concerns and prejudices. Disease and medication ideas can be modified and treatment plans should be developed in collaboration with the patient and parents, based on common goals.[42,43] Many factors, not only behavioral but also treatment–related, can affect treatment adherence. Dosing complexity and factors relating to inhaler and spacer use are important components of adherence.[44] Beyond asthma self-management and education, no specific resource has proven consistently helpful. Large, well conducted studies using interventions to improve adherence are lacking. Since non-adherence is a potentially adjustable factor that has a major effect on morbidity and mortality, research to determine better interventions should be done, rather than more studies comparing drugs and dosages. An effective partnership between physician and patient, to optimize adherence, and a personal asthma management plan should be the basis of asthma management for every child. This is in line with the recently developed National Asthma Standards of Care in The Netherlands.[45] Our findings suggest that further improvement may well be possible by taking individual phenotypic characteristics into account, and adapting the treatment plans accordingly.

Future research

Almost 95% of the children with asthma have mild to moderately severe asthma. We would focus on these patients because there is room for improvement of asthma control. Several lines of future research could help in achieving better asthma control:

- The most effective way to get better control is perhaps by improving selfmanagement of asthma and adherence to treatment. For this purpose optimizing communication between physicians and patients and parent is a key factor. Self management plans are mainly based on symptoms. For that reason we recommend future research efforts to focus on improving perception of bronchoconstriction, for example by immediate feedback on home spirometry, not only when symptoms are experienced but also during symptom-free periods. As optimal self-management is crucial for each asthma patient, the results of these studies should be incorporated in research trials on better asthma control.

- However even with excellent self-management and adherence there will be patients with uncontrolled moderately severe asthma and/or patients at risk for worse asthma outcome. These children, who are already treated with daily ICS, show highly variable individual responses on different treatment regimes. At this moment we cannot reliably predict which treatment regime will sort the best effect in a given patient. Hence, research on predictors of response to treatment and biomarkers to monitor asthma control is important. Because asthma is a heterogeneous disease it is naïve to think that a single biomarker would be sufficient to phenotype asthma patients. Selecting markers that are specific for clusters would therefore make sense.
- Before investigating treatment regimes on specific asthma phenotypes we should assess whether the phenotype is stable or not and at what time re-phenotyping is needed.
- The optimal frequency of various monitoring techniques should be established. It would for instance be useful to know if the outcome of the trial would differ with different monitoring intervals, e.g. not 3 but 6 monthly measurement of AHR. It is quite likely that optimal monitoring frequencies will depend on the nature and severity of the disease process.
- Long term effects of the AHR strategy as employed in the CAT study (and of other monitoring programmes) should be investigated, especially in children who have a phenotype that benefits most from a given regime.
- To overcome the problem of phenotype instability efforts should be made to investigate the relevance of endotypes, their stability and their usefulness for asthma monitoring and treatment.

The main challenge for the future will be to find successful and feasible combinations of biomarkers and other tools to establish phenotypes and/or endotypes of children who wheeze, in order to offer them a personalized therapy for achieving the best possible, cost-effective asthma outcome.

REFERENCES

1. Rabe KF, Adachi M, Lai CK, Soriano JB, Vermeire PA, Weiss KB, Weiss ST. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. The Journal of allergy and clinical immunology. 2004;114:40-7
2. Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2011. Available from: http://www.ginasthma.org/
3. Scott L, Morphew T, Bollinger ME, Samuelson S, Galant S, Clement L, O'Cull K, Jones F, Jones CA. Achieving and maintaining asthma control in inner-city children. The Journal of allergy and clinical immunology. 2011;128:56-63
4. Janson SL, McGrath KW, Covington JK, Cheng SC, Boushey HA. Individualized asthma self-management improves medication adherence and markers of asthma control. The Journal of allergy and clinical immunology. 2009;123:840-6
5. de Blic J, Boucot I, Pribil C, Robert J, Huas D, Marguet C. Control of asthma in children: still unacceptable? A French cross-sectional study. Respiratory medicine. 2009;103:1383-91
6. Kuehni CE, Frey U. Age-related differences in perceived asthma control in childhood: guidelines and reality. Eur Respir J. 2002;20:880-9
7. Wildhaber J, Carroll WD, Brand PL. Global impact of asthma on children and adolescents' daily lives: the room to breathe survey. Pediatric pulmonology. 2012;47:346-57
8. van Gent R, van Essen-Zandvliet LE, Rovers MM, Kimpen JL, de Meer G, van der Ent CK. Poor perception of dyspnoea in children with undiagnosed asthma. Eur Respir J. 2007;30:887-91
9. Siersted HC, Boldsen J, Hansen HS, Mostgaard G, Hyldebrandt N. Population based study of risk factors for underdiagnosis of asthma in adolescence: Odense schoolchild study. BMJ (Clinical research ed. 1998;316:651-5; discussion 5-6
10. Paterson NA, Peat JK, Mellis CM, Xuan W, Woolcock AJ. Accuracy of asthma treatment in schoolchildren in NSW, Australia. Eur Respir J. 1997;10:658-64
11. Caudri D, Wijga AH, Smit HA, Koppelman GH, Kerkhof M, Hoekstra MO, Brunekreef B, de Jongste JC. Asthma symptoms and medication in the PIAMA birth cohort: evidence for under and overtreatment. Pediatr Allergy Immunol. 2011;22:652-9
12. Stern G, de Jongste J, van der Valk R, Baraldi E, Carraro S, Thamrin C, Frey U. Fluctuation phenotyping based on daily fraction of exhaled nitric oxide values in asthmatic children. The Journal of allergy and clinical immunology. 2011;128:293-300
13. [1] Frey U, Suki B. Complexity of chronic asthma and chronic obstructive pulmonary disease: implications for risk assessment, and disease progression and control. Lancet. 2008;372:1088-99
14. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH. Cluster analysis and clinical asthma phenotypes. American journal of respiratory and critical care medicine. 2008;178:218-24
15. Brouwer AF, Roorda RJ, Brand PL. Home spirometry and asthma severity in children. Eur Respir J. 2006;28:1131-7

16. Uwyyed K, Springer C, Avital A, Bar-Yishay E, Godfrey S. Home recording of PEF in young asthmatics: does it contribute to management? Eur Respir J. 1996;9:872-9
17. Kamps AW, Roorda RJ, Brand PL. Peak flow diaries in childhood asthma are unreliable. Thorax. 2001;56:180-2
18. Wensley D, Silverman M. Peak flow monitoring for guided self-management in childhood asthma: a randomized controlled trial. American journal of respiratory and critical care medicine. 2004;170:606-12
19. Bentley AM, Menz G, Storz C, Robinson DS, Bradley B, Jeffery PK, Durham SR, Kay AB. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Relationship to symptoms and bronchial responsiveness. The American review of respiratory disease. 1992;146:500-6
20. Turato G, Barbato A, Baraldo S, Zanin ME, Bazzan E, Lokar-Oliani K, Calabrese F, Panizzolo C, Snijders D, Maestrelli P, Zuin R, Fabbri LM, Saetta M. Nonatopic children with multitrigger wheezing have airway pathology comparable to atopic asthma. American journal of respiratory and critical care medicine. 2008;178:476-82
21. Fleming L, Wilson N, Regamey N, Bush A. Use of sputum eosinophil counts to guide management in children with severe asthma. Thorax. 2012;67:193-8
22. Gibson PG, Simpson JL, Hankin R, Powell H, Henry RL. Relationship between induced sputum eosinophils and the clinical pattern of childhood asthma. Thorax. 2003;58:116-21
23. Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, Bradding P, Wardlaw AJ, Pavord ID. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax. 2007;62:1043-9
24. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. Lancet. 1999;353:2213-4
25. Lex C, Jenkins G, Wilson NM, Zacharasiewicz A, Erin E, Hansel TT, Bush A, Payne DN. Does sputum eosinophilia predict the response to systemic corticosteroids in children with difficult asthma? Pediatric pulmonology. 2007;42:298-303
26. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology (Carlton, Vic. 2006;11:54-61
27. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet. 2008;372:1107-19
28. Stanford RH, Gilsenan AW, Ziemiecki R, Zhou X, Lincourt WR, Ortega H. Predictors of uncontrolled asthma in adult and pediatric patients: analysis of the Asthma Control Characteristics and Prevalence Survey Studies (ACCESS). J Asthma. 2010;47:257-62
29. Kruzick T, Covar RA, Gleason M, Cicutto L, White M, Shocks D, Szefler SJ. Does access to care equal asthma control in school-age children? The Journal of allergy and clinical immunology. 2009;124:381-3
30. McQuaid EL, Kopel SJ, Klein RB, Fritz GK. Medication adherence in pediatric asthma: reasoning, responsibility, and behavior. Journal of pediatric psychology. 2003;28:323-33
31. Ducharme FM, Noya FJ, Allen-Ramey FC, Maiese EM, Gingras J, Blais L. Clinical effectiveness of inhaled corticosteroids versus montelukast in children with asthma: prescription patterns and patient adherence as key factors. Current medical research and opinion. 2012;28:111-9
32. Milgrom H, Bender B, Ackerson L, Bowry P, Smith B, Rand C. Noncompliance and treatment failure in children with asthma. The Journal of allergy and clinical immunology. 1996;98:1051-7

33. Bender B, Milgrom H, Rand C, Ackerson L. Psychological factors associated with medication nonadherence in asthmatic children. J Asthma. 1998;35:347-53
34. Reiser J, Warner JO. The value of participating in an asthma trial. Lancet. 1985;1:206-7
35. Mushlin AI, Appel FA. Diagnosing potential noncompliance. Physicians' ability in a behavioral dimension of medical care. Archives of internal medicine. 1977;137:318-21
36. Bender BG, Bartlett SJ, Rand CS, Turner C, Wamboldt FS, Zhang L. Impact of interview mode on accuracy of child and parent report of adherence with asthma-controller medication. Pediatrics. 2007;120:e471-7
37. Penza-Clyve SM, Mansell C, McQuaid EL. Why don't children take their asthma medications? A qualitative analysis of children's perspectives on adherence. J Asthma. 2004;41:189-97
38. Christiaanse ME, Lavigne JV, Lerner CV. Psychosocial aspects of compliance in children and adolescents with asthma. J Dev Behav Pediatr. 1989;10:75-80
39. Irvine L, Crombie IK, Alder EM, Neville RG, Clark RA. What predicts poor collection of medication among children with asthma? A case-control study. Eur Respir J. 2002;20:1464-9
40. Apter AJ, Reisine ST, Affleck G, Barrows E, ZuWallack RL. Adherence with twice-daily dosing of inhaled steroids. Socioeconomic and health-belief differences. American journal of respiratory and critical care medicine. 1998;157:1810-7
41. Bond M. Decision-making: Risk school. Nature. 2009;461:1189-92
42. Klok T, Brand PL, Bomhof-Roordink H, Duiverman EJ, Kaptein AA. Parental illness perceptions and medication perceptions in childhood asthma, a focus group study. Acta Paediatr. 2011;100:248-52
43. Gustafsson PM, Watson L, Davis KJ, Rabe KF. Poor asthma control in children: evidence from epidemiological surveys and implications for clinical practice. International journal of clinical practice. 2006;60:321-34
44. Murphy KR. Adherence to inhaled corticosteroids: comparison of available therapies. Pulmonary pharmacology & therapeutics. 2010;23:384-8
45. Zorgstandaard Asthma bij kinderen. Long Alliantie Nederland

Chapter 8

8

8 Summary

SUMMARY

Presently, asthma treatment is mainly based on symptoms. The preferred treatment strategy aims to reduce chronic airway inflammation, and it has been shown that symptoms do not accurately reflect this inflammation. In this thesis we investigated a new phenotype-driven treatment strategy for childhood asthma, not only based on symptoms but also based on airway hyperresponsiveness. We compared the results of this strategy to a treatment strategy based on symptoms only. We also assessed whether or not the benefits of the new treatment strategy persisted during the years after treatment was changed to usual care. To report symptoms, a child should be able to perceive and recognize airway obstruction. Clearly, inappropriate symptom perception would be a major problem for symptom-based treatment. Therefore we examined the accuracy of perception of bronchoconstriction in our study population. Obviously, biomarkers of asthmatic airway inflammation would be desirable as they may provide an objective means to assess disease activity.
During the CATO study, we repeatedly measured urinary eosinophil protein X, (uEPX), a marker of eosinophilic activation, that is completely non-invasive to collect and is potentially useful for asthma management in children, and examined the relation between uEPX levels and more conventional measures of asthma control.

Chapter 1 is a general introduction to phenotypes in childhood asthma and phenotype-driven asthma treatment strategies. It provides the aims of the studies of this thesis.

Chapter 2 describes the Childhood Asthma Treatment Optimal (CATO) study: a prospective multicentre, double-blinded 2-yr-intervention trial in 210 school-children with moderately severe asthma. We demonstrated that a treatment strategy driven by airway hyperresponsiveness (AHR-strategy) was more effective on lung function than a treatment strategy driven by symptoms. Lung function, expressed as the forced expiratory volume in 1 second (FEV_1), remained stable during 2 years of treatment based on the AHR-strategy, whereas lung function decreased in the control group. The positive effect on lung function was most obvious in a large, predefined subgroup of children characterized by low symptom scores despite AHR. The mean daily dose inhaled corticosteroids was significantly higher in the AHR-strategy group.

Symptom scores and AHR improved significantly and similarly with both treatment strategies. We concluded that 2-years of phenotype-driven asthma treatment was superior in terms of preservation of lung function.

Chapter 3 reports the results of the prospective follow up study of CATO (CATO-FU). One hundred and thirty-seven children of the original study population participated. The mean interval between the last CATO visit and first CATO-FU visit was 4,4 years. During this period, asthma treatment was managed by the pediatrician or the family doctor according to national guidelines. We wanted to investigate whether the positive effect on lung function, as observed in the CATO study, was sustained during these years after the intervention period. We found that symptom scores, lung function and asthma exacerbation rates were similar for both treatment strategy groups, while AHR had not changed. The mean daily dose of inhaled corticosteroids had been reduced by 50%. We concluded that the beneficial effect on lung function of 2 years treatment guided by AHR was lost after 4,4 yrs of usual care. This suggest that an AHR-driven treatment strategy may need to be sustained in order to preserve lung function on the long term.

Chapter 4 presents our study of perception of bronchoconstriction in the CATO study population. Perception was measured by Borg scores during a methacholine bronchoprovocation test on one or two study visits. We found that a poorer perception was associated with AHR, lower baseline lung function and less use of short-acting bronchodilators as rescue medication. We reasoned that poor perception of bronchoconstriction will influence symptom scores, probably leading to undertreatment. Therefore we suggested that measurements of symptom perception should be taken into account in individual management plans for children with asthma.

Chapter 5 describes cross-sectional relations between urinary eosinophil protein X (uEPX) and conventional markers of asthma severity collected in 180 children participating in the CATO study. We found an inverse correlation between uEPX and lung function, and no correlation between uEPX and symptoms or other markers of eosinophilic inflammation such as percentage eosinophils in sputum and nitric oxide in exhaled air. We conclude that only lung function was correlated to uEPX in this cross-sectional study, but that the correlation was weak and numbers small. These finding were not encouraging for uEPX as a complementary marker of eosinophilic airway inflammation in asthma.

In **chapter 6** we further explored the usefulness of uEPX as a marker of disease and eosinophilic airway inflammation. During the CATO study, at baseline, after one year and after 2 years urine samples were collected and assessed on EPX levels. The changes in uEPX over time were examined and associated with changes in symptom scores, lung function and eosinophils in induced sputum in the same interval. uEPX levels decreased significantly during the study. We found positive correlations for changes in uEPX and changes in lung function and for changes in uEPX and changes in sputum eosinophils, albeit with a large scatter. This suggests that repeated measures of uEPX are not very useful as a marker of disease activity and eosinophilic airway inflammation for the individual pediatric patient.

In **chapters 7** we present a general discussion of phenotype-driven asthma treatment and recommendations for future research.

SAMENVATTING

In de dagelijkse praktijk wordt de behandeling van astma voornamelijk gestuurd door de ernst van gerapporteerde symptomen. De behandeling is gericht op het verminderen van chronische ontsteking in de luchtwegen: ontstekingsremmende medicijnen zijn het meest effectief bij de behandeling van astma. Het is echter aangetoond dat de ernst van symptomen niet correleert met de ernst van de ontsteking in de luchtwegen. In dit proefschrift wordt beschreven hoe een nieuwe fenotypegestuurde behandelstrategie voor kinderen met astma werd onderzocht. Het vernieuwende van deze strategie is dat de behandeling niet alleen gebaseerd is op symptomen maar ook op abnormale prikkelbaarheid van de luchtwegen (airway hyperresponsiveness = AHR). We vergeleken deze nieuwe strategie met de gebruikelijke symptoomgebaseerde behandelstrategie (Childhood Asthma Treatment Optimal (CATO studie)). Ook onderzochten we of de voordelen van de nieuwe behandelstrategie nog waarneembaar waren, nadat de kinderen al weer jarenlang op de traditionele manier werden behandeld. Om astmaklachten, waaronder benauwdheid, aan te kunnen geven, moet een kind in staat zijn luchtwegvernauwing te herkennen. Het mag duidelijk zijn dat onjuiste perceptie van symptomen een groot probleem is bij een behandelstrategie die alleen gebaseerd is op symptomen. Daarom werd de accuraatheid van het herkennen (perceptie) van luchtwegvernauwing onderzocht.
Lichaamseigen stoffen die de ernst van luchtwegontsteking weergeven (zogenaamde biomarkers) zouden nuttig kunnen zijn om de activiteit van de ziekte te meten. Gedurende de CATO-studie werd op gezette tijden 'eosinophil protein X' in een portie urine gemeten (uEPX). UEPX is een eiwit dat door geactiveerde eosinofiele granulocyen (ontstekingscellen die een grote rol spelen bij astma) wordt geproduceerd. Het meten van deze biomarker kan volkomen niet invasief gebeuren. Omdat uEPX waardevol zou kunnen zijn bij de behandeling van kinderen met astma werd de relatie tussen uEPX-waarden en de gebruikelijke parameters voor de mate van astmacontrole onderzocht.

Hoofdstuk 1 is een algemene inleiding over astma op de kinderleeftijd en de verschillende fenotypegestuurde behandelstrategieën. In dit hoofdstuk worden de doelen van het onderzoek uiteengezet.

Hoofdstuk 2 beschrijft de CATO-studie. De CATO-studie is een gerandomiseerd, dubbelblind onderzoek, waaraan 210 kinderen uit 15 ziekenhuizen in Nederland deelnamen. De gerandomiseerde kinderen, met matig ernstig astma, werden gedurende 2 jaar behandeld: per individu werd de behandeling gestuurd op grond van de ernst van de symptomen (referentiestrategie) ofwel werd de behandeling voornamelijk gestuurd door de mate van AHR (AHR-strategie). Laatstgenoemde, nieuwe behandelstrategie had gunstig effect op de longfunctie: gedurende de studie veranderde de gemiddelde longfunctie bij de kinderen die volgens de AHR strategie behandeld werden niet, terwijl de gemiddelde longfunctie bij de kinderen die behandeld werden volgens de referentiestrategie verslechterde. Dit effect op longfunctie was het meest duidelijk waarneembaar binnen een van tevoren gedefinieerde subgroep, namelijk de groep kinderen die aangaven weinig of geen last te hebben van hun astma (weinig symptomen), terwijl ze wel abnormaal prikkelbare luchtwegen hadden. De gemiddelde dosis ontstekingsremmende medicijnen in de groep kinderen die volgens de AHR-strategie werd behandeld was significant hoger dan de gemiddelde dosis die de kinderen in de referentiegroep nodig hadden. Klachtenscores en prikkelbaarheid van de luchtwegen verbeterden bij beide behandelstrategieën in gelijke mate.
Geconcludeerd werd dat een tweejarige behandeling gestuurd op mate van prikkelbaarheid van de luchtwegen superieur was ten opzichte van een behandeling gestuurd op klachten.

Hoofdstuk 3 beschrijft de resultaten van de prospectieve vervolgstudie na de CATO studie (CATO-FU). Honderdzevenendertig kinderen die eerder deel uitmaakten van de CATO studie participeerden in deze studie. De gemiddelde duur van de periode tussen CATO en CATO-FU bedroeg 4,4 jaar. Gedurende deze periode werden de kinderen behandeld door de kinder(long) arts of huisarts volgens de Nederlandse richtlijnen voor behandeling van kinderen met astma. De vraag van het vervolgonderzoek was of het gunstige effect van de AHR-strategie op de longfunctie nog steeds waarneembaar was jaren na het staken van deze behandelstrategie. Dit bleek niet het geval te zijn. Symptoomscores en het aantal astma aanvallen verschilden niet tussen de beide behandelstrategieën. De prikkelbaarheid van de luchtwegen gemeten tijdens CATO-FU was niet veranderd ten opzichte van het einde van de CATO-studie. De gemiddelde dosis ontstekingsremmende medicijnen was gehalveerd ten opzichte van het eind van de CATO-studie.

Geconcludeerd werd dat het gunstige effect van de tweejarige AHR-behandelstrategie niet meer aanwezig was, nadat de kinderen gedurende 4,4 jaar volgens de Nederlandse richtlijnen werden behandeld.

In **hoofdstuk 4** worden de resultaten beschreven van het onderzoek naar perceptie van luchtwegvernauwing. Een gedeelte van de kinderen die deelnamen aan de CATO-studie werd tijdens een of meerdere bezoeken gevraagd een Borgscore in te vullen ten tijde van de methacholineprovocatietest (test om de mate van prikkelbaarheid van de luchtwegen te meten). Methacholine is een stof die, na inademing, bij de meeste mensen met astma leidt tot luchtwegvernauwing. Tijdens een methacholineprovocatietest inhaleert een kind methacholine in oplopende dosis (verdubbelingstappen), na elke dosis wordt de longfunctie gemeten. De Borgscore is een gestandaardiseerde verticale lijst met een schaalverdeling 0-10 overeenkomend met geen benauwdheid - meest ernstige benauwdheid ooit. De Borg- score wordt vergeleken met de mate van luchtwegvernauwing die door middel van longfunctie gemeten werd. Het werd duidelijk dat een slechtere herkenning van luchtwegvernauwing samenhing met meer hyperreactiviteit van de luchtwegen, een lagere uitgangslongfunctie. Kinderen met slechtere perceptie gebruikten bovendien minder kortwerkende luchtwegverwijders (medicijnen om onverwachte benauwdheidklachten te behandelen).
Geconcludeerd werd dat deze resultaten suggereren dat het meten van de perceptie van luchtwegvernauwing belangrijk is bij het maken van individueel behandelplan voor een kind met astma.

Hoofdstuk 5 beschrijft de cross-sectionele relatie tussen uEPX en de gebruikelijke parameters van de ernst van astma bij 180 kinderen die deelnamen aan de CATO-studie. Er werd een omgekeerde relatie tussen uEPX en longfunctie gevonden, geen correlatie tussen uEPX en symptomen of markers van eosinophiele luchtwegontsteking, zoals het percentage eosinophiele granulocyten in sputum of NO in de uitademingslucht.
We concludeerden dat alleen longfunctie gecorreleerd was aan uEPX in dit cross-sectionele onderzoek, maar dat de correlatie zwak was en het aantal onderzochte kinderen vrij klein. De resultaten gaven weinig reden te denken dat uEPX geschikt is als aanvullende marker van eosinophiele ontsteking in de luchtweg bij kinderen met astma.

Hoofdstuk 6 beschrijft verder onderzoek naar de waarde van uEPX als parameter van astma en eosinophiele luchtwegontsteking. Tijdens de CATO-studie, bij het begin, na 1 en 2 jaar werden urinemonsters verzameld, hierin werden EPX-spiegels gemeten. De veranderingen in de waarden van uEPX gedurende de studie werden vergeleken met de veranderingen in symptoomscores, longfunctie en eosinophiele granulocyten in sputum. UEPX-spiegels namen significant af tijdens de studie. Er waren positieve correlaties tussen veranderingen in uEPX en de veranderingen in longfunctie en veranderingen in uEPX en veranderingen in sputum eosinophielen, hoewel de spreiding groot was. Dit suggereert dat ook herhaalde metingen van uEPX niet waardevol zijn als parameter om de activiteit van astma en/of eosinophiele luchtwegontsteking te meten bij individuele astmapatiënten.

Hoofdstuk 7 geeft een algemene discussie over fenotypegestuurde behandeling van astma bij kinderen en aanbevelingen voor toekomstig wetenschappelijk onderzoek op het terrein van astma op de kinderleeftijd.

DANKWOORD

Eindelijk is het dan zover: ik mag - last but not least - het dankwoord schrijven. Dit is het moment om iedereen te bedanken die heeft geholpen de studies uit te voeren, de artikelen en het proefschrift te schrijven.

Allereerst de kinderen en hun ouders die aan de studies hebben deelgenomen. Alle uren die jullie op de longfunctieafdeling doorbrachten, de dagboekjes die jullie invulden, en nog veel meer, het was allemaal onmisbaar!

Professor J.C. de Jongste, beste Johan, bedankt voor je niet-aflatende steun en begeleiding. Zonder jouw motiverende woorden en opbouwende commentaar was dit boekje er niet geweest.
Professor E.J. Duiverman, beste Eric, bij jou in het Juliana Kinderziekenhuis begon ik met wetenschappelijk onderzoek en de opleiding tot kinderlongarts. Ondanks je vertrek naar het Groningse bleef je me begeleiden. Fijn dat je ondanks de afstand toch mijn promotor wilde blijven.
Promotoren, mijn dank is heel groot.

Professor P.J. Sterk, beste Peter, aan het begin van mijn wetenschappelijke carrière, in de tijd dat je nog in Leiden werkte, kwam ik daar regelmatig over de vloer. Van de researchbesprekingen heb ik heel veel geleerd. Het stuk "the joy of writing a paper" was zeker aan mij besteed. Bedankt voor je enorme inzet.

Kinder(long)artsen, longfunctie analisten, kinderlongverpleegkundigen en research verpleegkundigen van de participerende ziekenhuizen. Dank voor het vele werk dat jullie verzet hebben en voor de hartelijke ontvangsten tijdens de 'CATO-site-visits'!

Tijdens deze 'site-visits' was Jan Boogaard bijna altijd van de partij, altijd geïnteresseerd, in voor een goede discussie en goedgehumeurd. Ik vind het erg jammer dat hij niet meer bij mijn promotie aanwezig kan zijn.

Anja, ik begreep al snel nadat ik was aangesteld, dat jij mij voor was gegaan in soortgelijk wetenschappelijk onderzoek. Dank voor je grote inzet, in het bijzonder voor de CATO follow-up studie. Aan de ontmoeting bij jou thuis, waar steeds meer grijze ordners op tafel verschenen en aan de lekkere broodjes bij het tentje even verderop, heb ik goede herinneringen.

GlaxoSmithKline, in het bijzonder Pauline en Joke, bedankt voor jullie hulp met de gigantische 'papierhandel', het regelen van vergaderingen en bijeenkomsten en zoveel meer!

Wim, tijdens mijn opleiding heb ik statistiek gehad, aan de Erasmus Universiteit maar liefst, daarna heb ik nog een cursus gevolgd. Wat er allemaal kwam kijken bij de CATO-studie, had ik desondanks niet kunnen bedenken! Dank voor al je inspanningen!

Nellie, Marianne en Jeane, (ex-)longfunctieanalisten van het Juliana Kinderziekenhuis, wat een kanjers zijn jullie. Door dik en dun, altijd kreeg ik jullie steun! Bedankt voor de fantastische samenwerking!

Jan, je was niet alleen actief betrokken bij de CATO-studie, van jou heb ik na mijn fellowship in het academische geleerd hoe je als kinderlongarts werkt in een groot niet-academisch ziekenhuis. Door de belspreekuren die je in 'ons hok' deed, besefte ik elke keer weer: als je zelf weinig woorden gebruikt, heb je meer tijd om te luisteren!
Arwen, de rondleiding die ik op mijn eerste werkdag van je kreeg, kan ik me nog steeds herinneren. Ik begreep niet hoe je in dat gebouw de weg kon vinden! Zo heb je me bij meer wegwijs gemaakt: het korte gesprekje over wetenschappelijk onderzoek doen, zonder promoveren, is nog vaak in mijn hoofd naar boven gekomen! Ik ben blij dat je je hart verloren hebt aan de kinderlongziekten! Jan en Arwen, bedankt voor de invalmomenten en hulp.

Lieve collegae, kinderartsen van het JKZ, te veel om allemaal apart te noemen (dan zou het toch nog een dik boek worden), door jullie collegialiteit, professionaliteit, relativerend vermogen en humor is werken in het Juliana Kinderziekenhuis, het beste wat een kinderarts kan overkomen. Zelfs promoveren is dan mogelijk. Bedankt!
In het bijzonder wil ik hier Frederique en Frank bedanken. Waar een walk around al niet goed voor is! Jullie hebben me het laatste zetje gegeven en de middelen aangereikt om dit boekje af te maken.

Mariëlle, wat ben ik blij dat je mijn paranimf bent! Vanaf het moment dat we met elkaar opgescheept zaten in het Sophia Kinderziekenhuis, klikte het. Je warme persoonlijkheid en je groot invoelend vermogen zijn me dierbaar! Ik hoop dat het altijd zo blijft!
Iris, niet alleen kamergenoot maar ook paranimf, dank dat je beide taken wilt vervullen! Wat zijn we snel aan elkaar gewend geraakt op onze kamer waar ik nu de plek bij het raam heb. Ik ben blij dat je in het Juliana Kinderziekenhuis bent komen werken, nog blijer dat dat op de kinderlongziekten is!

Lieve papa en mama, wat vind ik het fijn dit boekje aan jullie te mogen opdragen. Het veilige nest dat jullie ons geboden hebben en de mogelijkheid ons te ontwikkelen, waren onmisbaar. Ik wil jullie daarvoor bedanken, alsook voor al die keren dat jullie naar Den Haag kwamen om op de kinderen te passen, de tuin onderhanden namen en nog zoveel meer. Beter kon het niet!

Sophie, Anna en Suzanne, allerliefste meisjes van de hele wereld, wat maken jullie ons leven mooi! Sophie, meteen wist je hoe prikkelbare longen/luchtwegen eruitzien. Dank je wel dat ik jouw tekening op de voorkant van dit boekje mocht zetten.
Lieve Roel, rots in de branding, dank voor je onvoorwaardelijke steun en vertrouwen. Zonder jou was dit 'project' niet tot een goed einde gekomen. Er is zoveel meer moois in leven dan wetenschap en werken, ik wil niets liever dan hier samen met jou en de kinderen nog heel lang van genieten.

CURRICULUM VITAE

Marianne Nuijsink was born in Rijssen on May 3rd, 1967. She passed her secondary school at "College Noetsele " in Nijverdal in 1985. In the same year she started her medical training at the Medical Faculty of the Erasmus University Rotterdam. After obtaining her Medical Degree in 1991 she worked as a resident in Pediatrics in Utrecht (Wilhelmina Children's Hospital). In 1993 she started her specialist training in Pediatrics at the Wilhelmina Children's Hospital in Utrecht (head Prof. dr. JJ Stoop/Prof. dr. A. Okken) and finished it in 1998 in the Juliana Children's Hospital-HAGA Teaching Hospital in The Hague (head Prof. dr. A.J. van der Heijden). In the same year and the same hospital she started her training as a fellow in Paediatric Pulmonology, under supervision of dr. Eric Duiverman. When dr. Duiverman became Professor in Pediatric Pulmonology at the University Medical Centre Groningen in 1999, Prof. Johan de Jongste became her supervisor and her fellowship was continued in the Erasmus MC-Sophia Children's Hospital Rotterdam. The research she performed during her fellowship and thereafter is presented in this thesis. GlaxoSmithKline funded this fellowship and research program. Since 2001 she is staff member of the department Pediatrics in the Juliana Children's Hospital-HAGA Teaching Hospital.

She is married with Roel Jacobs, who is a golf professional, and together they have three daughters Sophie (2003), Anna (2004) and Suzanne (2006).

Chapter 9

Addendum

Behandeling van astma bij kinderen op geleide van bronchiale hyperreactiviteit

Marianne Nuijsink, Wim C.J. Hop, Peter J. Sterk,
Eric J. Duiverman en Johan C. de Jongste

Ned Tijdschr Geneeskd. 2009

9 Addendum
Behandeling van astma bij kinderen op geleide van bronchiale hyperreactiviteit

SAMENVATTING

Doel
Onderzoeken of bij kinderen met astma een behandelstrategie op geleide van symptomen én bronchiale hyperreactiviteit van de luchtweg (BHR) het aantal symptoomvrije dagen doet toenemen en de longfunctie verbetert in vergelijking met een behandelingstrategie op geleide van symptomen alleen.

Opzet
Multicentrisch dubbelblind gerandomiseerd interventieonderzoek met parallelgroep gedurende 2 jaar.

Methode
210 kinderen (leeftijd 6-16 jaar) met matig ernstig atopisch astma, geselecteerd op grond van symptoomscores en aanwezigheid van BHR, werden gerandomiseerd voor een van de 2 behandelstrategieën. Elke 3 maanden werden symptoomscores, het geforceerde expiratoire 1-secondevolume (FEV_1) en BHR (uitgedrukt in PD_{20}: de dosis die bij de methacholineprovocatietest een afname van 20% in het FEV_1 geeft) gemeten. Daarna werd de medicatie (fluticason 100-1000 µg /dag, eventueel met salmeterol 100 µg /dag) aangepast volgens een algoritme gebaseerd op de symptoomscores alleen (referentiestrategie, n = 104) of de mate van BHR én symptoomscores (BHR-strategie, n = 102).

Resultaten
Na 2 jaar werd tussen de behandelstrategieën geen verschil in het percentage symptoomvrije dagen gevonden. Bij kinderen behandeld volgens de BHR-strategie was het FEV_1 significant hoger dan bij kinderen behandeld volgens de referentiestrategie (verschil 2,3% van de voorspelde waarde; p = 0,046). Dit verschil werd verklaard door een geleidelijke afname van het FEV_1 in de referentiestrategie in een subgroep van kinderen met BHR en weinig klachten bij randomisatie. Aan het einde van de studie was het verschil in FEV_1 6% tussen beide behandelingsstrategieën in deze subgroep (n = 91; p = 0,017).

Conclusie
Astmabehandeling op geleide van BHR verbeterde het percentage symptoomvrije dagen niet, maar voorkwam longfunctieverlies bij allergisch astmatische kinderen, vooral bij kinderen met weinig symptomen ondanks evidente BHR.

INTRODUCTIE

De prognose van astma hangt af van de ernst van de ziekte op jonge leeftijd: de kans dat de ziekte aanhoudt op volwassen leeftijd is groter bij kinderen met een ernstiger vorm van luchtwegobstructie en bronchiale hyperreactiviteit.[1] Onlangs is aangetoond dat astma gepaard gaat met een verminderde longfunctie op jongvolwassen leeftijd.[2] Daarom zou bij kinderen een behandelstrategie die, naast symptoomcontrole, de longfunctie op de kinderleeftijd verbetert, op lange termijn aanzienlijke voordelen kunnen bieden.

Ondanks effectieve behandeling is de astmacontrole bij grote groepen astmapatiënten verre van optimaal.[3] Eén van de verklaringen is dat de ontsteking van de luchtweg onvoldoende wordt onderdrukt. Inhalatiecorticosteroïden (ICS) zijn effectief in het onderdrukken van deze ontsteking en eerste keus bij de behandeling voor persisterend astma.[4] ICS worden volgens de richtlijnen van het 'Global initiative for asthma' (GINA) aangepast op geleide van symptomen en de longfunctie.[5] Het verband tussen symptomen, longfunctie en de ernst van luchtwegontsteking is echter zwak of ontbreekt.[6] Ondanks behandeling met ICS kan ontsteking in de luchtwegen persisteren, zelfs bij astmapatiënten in klinische remissie.[7,8] Dit betekent dat men bij de behandeling van astma niet alleen met symptomen of longfunctie, maar ook met luchtwegontsteking rekening moet houden. Onderzoek met verschillende behandelstrategieën bevestigt de validiteit van dit concept.[9–12]

Hyperreactiviteit van de luchtweg is het gevolg van ontsteking en remodellering; dit laatste is het verdikken en verstijven van de luchtweg ten gevolge van karakteristieke structurele veranderingen ervan.[7,13] Bij volwassen astmapatiënten is reeds aangetoond dat door titratie van ICS op geleide van bronchiale hyperreactiviteit (BHR) het aantal astma-aanvallen en de remodellering afnamen en de longfunctie verbeterde.[14] BHR is een risicofactor voor snel longfunctieverlies.[1] De mate van BHR hangt samen met de perceptie van de bronchoconstrictie door de patiënt: ernstige BHR gaat gepaard met onvoldoende symptoomperceptie.[15,16] Dit kan betekenen dat er een risico is op snellere

achteruitgang van longfunctie bij kinderen met hyperreactieve luchtwegen als de medicatie alleen wordt aangepast op basis van de symptomen. Deze kinderen zouden baat kunnen hebben bij titratie van astmatherapie op geleide van BHR.

Wij verrichtten een onderzoek om te toetsen of een behandelstrategie op geleide van BHR resulteert in meer symptoomvrije dagen en een betere longfunctie dan een behandelstrategie op geleide van symptomen. Het onderzoek werd uitgevoerd bij kinderen met matig ernstig atopisch astma.

PATIËNTEN EN METHODEN

Patiënten

Kinderen (6–16 jaar) met een klinische voorgeschiedenis van matig ernstig allergisch astma (volgens de GINA-richtlijnen) werden geselecteerd door kinder(long)artsen in de tweede en de derde lijn. Zij gebruikten minimaal 200 µg fluticason per dag of een equivalente dosis van een van de andere ICS. Om in aanmerking te komen voor deelname aan de studie werd gebruikgemaakt van de cumulatieve symptoomscore, gemeten gedurende de laatste 2 weken voor randomisatie (tabel 1) en de mate van bronchiale hyperreactiviteit (BHR).

Tabel 1 *- Symptoomscore in een studie naar behandelstrategieën van matig ernstige astma bij kinderen*

Score astmaklachten tijdens de afgelopen nacht (iedere ochtend invullen)	
0	geen hoesten, piepen en kortademigheid
1	hoesten, piepen of kortademigheid waardoor je 1 keer wakker werd of vroeg wakker bent geworden
2	hoesten, piepen, of kortademigheid waardoor je twee keer of meer wakker bent geworden (inclusief vroeg wakker worden)
3	zoveel hoesten, piepen, of kortademigheid dat je niet geslapen hebt

Score astmaklachten overdag (iedere avond invullen)	
0	geen last van hoesten, piepen en kortademigheid
1	gehoest, gepiept of kortademig geweest gedurende 1 korte periode op de dag
2	klachten van hoesten, piepen of kortademigheid gedurende twee of meer korte periodes op de dag
3	gedurende (vrijwel) de gehele dag last van hoesten, piepen of kortademigheid

BHR werd gekwantificeerd met een methacholineprovocatietest. Deze test meet de dosis geïnhaleerd methacholine die nodig is om het geforceerde expiratoire 1-secondevolume (FEV_1) met 20% te laten afnemen (PD_{20}). Er werd onderscheid gemaakt tussen 3 verschillende fenotypen astma: (a) BHR-subgroep: kinderen met een sterke BHR en weinig symptomen (PD_{20} < 150 µg, cumulatieve symptoomscore < 14), (b) S-subgroep: kinderen met veel symptomen en een lage BHR (cumulatieve symptoomscore ≥ 14 en PD_{20} ≥ 150 µg) en (c) S+BHR-subgroep: kinderen met veel symptomen en een sterke BHR (cumulatieve symptoomscore ≥ 14 en PD_{20} < 150 µg). Wij onderscheidden deze verschillende fenotypen zodat de resultaten toepasbaar zouden zijn op het ziektespectrum binnen de praktijk.

Onderzoeksopzet

In een 2-jarig multicentrisch, prospectief dubbelblind gerandomiseerd interventieonderzoek met parallelle groepen werden, na een inloopperiode van 1 maand, de kinderen gerandomiseerd behandeld volgens een BHR- of een referentiestrategie. Bij de referentiestrategie werd de behandeling alleen op symptoomscore gebaseerd, terwijl bij de BHR-strategie de behandeling werd aangepast op geleide van de PD_{20} en de symptoomscore (tabel 2). Tijdens de inloopperiode werden patiënten ingesteld op 100 of 250 µg fluticason tweemaal daags, afhankelijk van de behandeling vóór de inloopperiode. Na randomisatie werd aan de ICS een langwerkende bèta-agonist (salmeterol) toegevoegd. De cumulatieve symptoomscore, longfunctie (FEV_1 en de geforceerde vitale capaciteit (FVC)) en BHR (PD_{20}) werden elke 3 maanden gemeten. Op basis van de uitkomsten van de symptoomscore of BHR werd de medicatie volgens het algoritme aangepast (tabel 2 en 3). Voor symptoomverlichting was salbutamol toegestaan.

Tabel 2 *- Medicatie niveaus in een studie naar behandelstrategieën van matig ernstige astma bij kinderen*

Niveau 1	100 µg fluticason 1 dd
Niveau 2	100 µg fluticason 2 dd
Niveau 3	100 µg fluticason-50 µg salmeterol 2 dd
Niveau 4	250 µg fluticason-50 µg salmeterol 2 dd
Niveau 5	500 µg fluticason-50 µg salmeterol 2 dd

Tabel 3 *- Behandel algoritme in een studie naar 2 behandelstrategieën van matig ernstige astma bij kinderen: gebaseerd op symptoomscores alleen (referentiestrategie) of op bronchiale hyperreactiviteit (BHR) en symptoomscores*

Medicatie niveau	Referentie strategie	BHR strategie
1 niveau verhogen	CSS ≥ 14	PD_{20} < 100 µg en SS < 14 of
		PD_{20} < 300 µg en SS ≥ 14
niveau handhaven	0 < CSS < 14	PD_{20} 100-300 µg en SS < 14 of
		PD_{20} ≥ 300 µg en SS ≥ 14
1 niveau verlagen	CSS = 0	PD_{20} > 300 µg en SS < 14

SS: cumulatieve symptoom score gedurende 14 dagen voor kliniekbezoek; PD_{20}: de provocatiedosis methacholine die een afname van 20% in het geforceerd expiratoir eensecondevolume geeft.

Uitkomstmaten

De primaire uitkomstmaat van de studie was het percentage symptoomvrije dagen in de laatste 3 maanden. Tijdens de inloopperiode, de 7 periodes van elk 14 dagen voorafgaand aan elk kliniekbezoek, en tijdens de laatste 3 maanden van het onderzoek werden dagboekgegevens verzameld.[17] Piepen, hoesten en kortademigheid 's nachts en overdag werden genoteerd op een 4-puntsschaal (0 = geen symptomen, 3 = ernstige symptomen die de activiteit of de slaap beïnvloeden). Voor elke periode werd het percentage symptoomvrije dagen bepaald, gedefinieerd als een score van 0 voor piepen, hoesten en kortademigheid.

Voor het verrichten van longfunctie- en BHR-onderzoek werd de onderzoeksmedicatie 36 h gestaakt. Bij elk bezoek werden FEV_1 en FVC gemeten.[18] BHR werd getest door provocatie met methacholine.[19] Een astma-aanval werd gedefinieerd als een toename van astmasymptomen die naar het oordeel van de behandelend arts behandeld moest worden met een orale corticosteroïdkuur.[20]

Veiligheidsmaatregelen

Elke 3 maanden werd de lichaamslengte gemeten. Als binnen 6 maanden bij 2 opeenvolgende bezoeken een afname van de groei van tenminste 0,125 SD werd waargenomen, vond verdere beoordeling door een onafhankelijke kinderendocrinoloog plaats. Bij een vermoeden op ernstige groeiproblemen kon de patiënt uit het onderzoek worden teruggetrokken.

Statistische analyse

Bij een tweezijdige α van 0,05 waren in elke arm 100 patiënten nodig om aan het einde van het onderzoek een verschil van 15% in het percentage symptoomvrije dagen aan te kunnen tonen met een onderscheidend vermogen ('power') van 80%. De powerberekening was gebaseerd op eerder onderzoek.[17] Symptoomvrije dagen en de longfunctie (FEV_1 (% voorspelde waarde), FEV_1/FVC x 100%) werden vergeleken in variantieanalyses voor herhaalde metingen (RmANOVA). PD_{20}-resultaten werden geanalyseerd na logaritmische transformatie. Het cumulatieve risico op astma-aanvallen gedurende de studie werd vergeleken met de logranktoets.

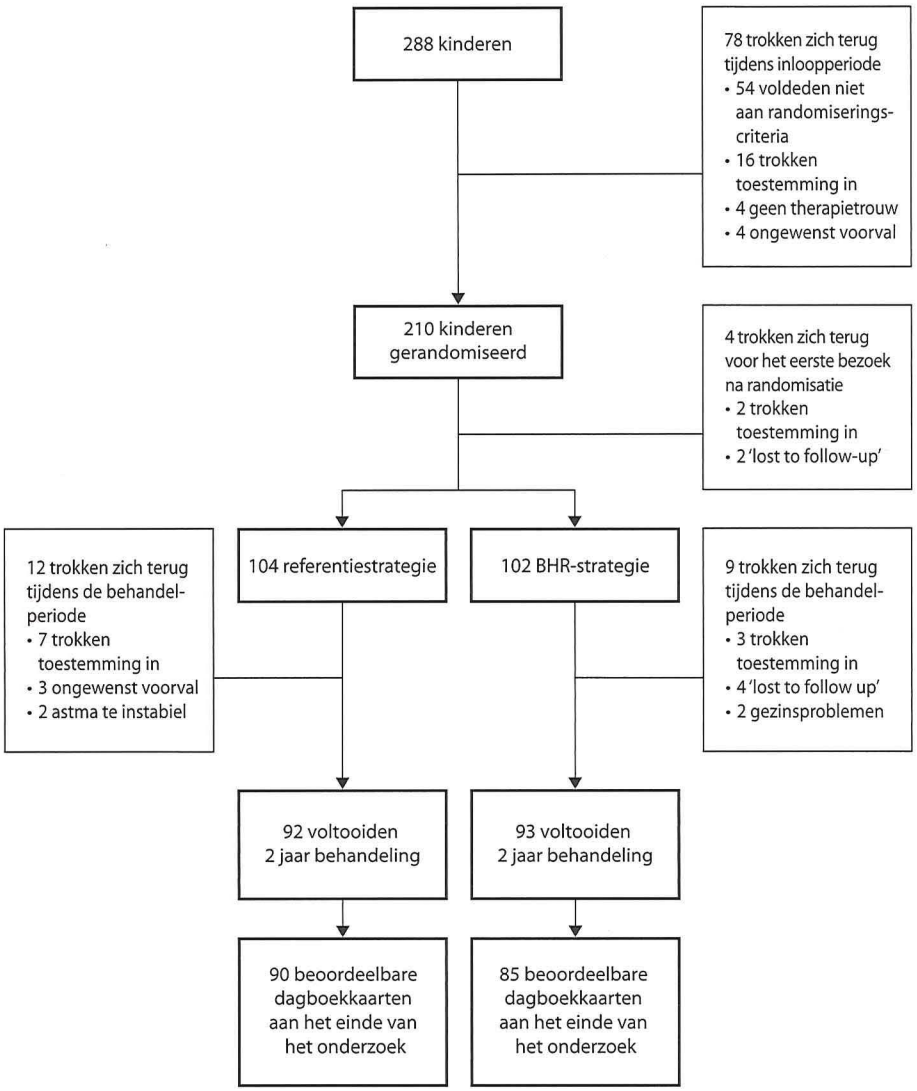
Alle analyses werden uitgevoerd met correctie voor een mogelijk relevante disbalans in het aantal exacerbaties in het jaar vóór het onderzoek. Voor de 3 geïncludeerde fenotypen werden vooraf geplande subgroepanalyses uitgevoerd. Alle analyses werden volgens het 'intention-to-treat'-principe uitgevoerd.

RESULTATEN

Patiënten

In de periode december 1999-oktober 2003 werden 288 kinderen geïncludeerd, van wie er 210 voldeden aan de criteria voor randomisatie. Aan het einde van de studie konden de resultaten bij 206 kinderen worden beoordeeld (figuur 1): 91 (44%) in de BHR-subgroep, 46 (22%) in de S-subgroep en 69 (34%) in de S+BHR-subgroep.

Figuur 1 *- Onderzoeksschema naar behandelstrategieën van matig ernstige astma bij kinderen; BHR = bronchiale hyperreactiviteit.*



De kinderen behandeld met de BHR-strategie hadden meer astma-exacerbaties gehad in het jaar vóór het onderzoek. Voor alle andere parameters waren er geen verschillen (tabel 4).

Tabel 4 *- Basiskenmerken van de deelnemers van een studie naar 2 behandelstrategieën van matig ernstige astma bij kinderen: gebaseerd op symptoomscores alleen (referentiestrategie) of op bronchiale hyperreactiviteit (BHR) en symptoomscores. BHR-subgroep= kinderen met BHR en goede symptoomcontrole tijdens inloopperiode van de studie; S-subgroep= kinderen met duidelijke symptomen zonder BHR tijdens de inloopperiode. S+BHR-subgroep: kinderen met duidelijke symptomen en BHR tijdens inloopperiode; ICS = inhalatiecorticosteroïden; FEV_1 = geforceerd expiratoir 1-secondevolume; FVC = geforceerde vitale capaciteit; PD_{20} = methacholinedosis die een afname van 20% in FEV_1 geeft*

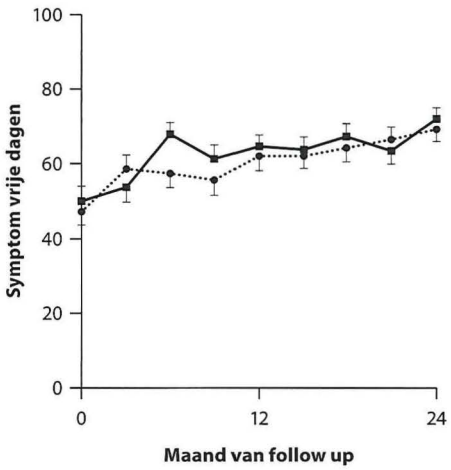
	Totale onderzoekspopulatie		**Subgroepen**		
	Referentie strategie (n = 104)	**BHR-strategie (n = 102)**	**BHR (n = 91)**	**S (n = 46)**	**S+BHR (n = 69)**
Geslacht (n, man/vrouw)	54/50	63/39	54/37	30/16	33/36
Leeftijd in jaren;gemiddelde (SD)	10,9 (2,5)	10,8 (2,4)	10,7 (2,5)	11,0 (2,1)	11,0 (2,5)
Duur van de astma in jaren; gemiddelde (SD)	7,1 (3,0)	7,3 (3,1)	7,3 (3,0)	7,0 (3,3)	7,3 (3,0)
Duur van ICS-gebruik in jaren; gemiddelde (SD)	5,2 (2,7)	5,8 (2,7)	5,4 (2,9)	5,4 (3,0)	5,7 (2,4)
Kinderen met ≥1 astma-aanval in het jaar voor het onderzoek; n(%)	12	25	20	5	12
FEV_1 in % voorspelde waarde; gemiddelde (SD)	98 (14)	96 (14)	97 (15)	100 (12)	94 (14)
FEV_1/FVC in%; gemiddeld (SD)	83 (8)	81(9)	81 (9)	86 (6)	81 (8)
PD_{20} in µg; mediaan (uitersten)	73 (0,8- >1570)	68 (0,8- >1570)	47 (3-148)	553 (154->1570)	42 (0,8-144)
Cumulatieve symptoomscore in de laatste 14 dagen van de inloopperiode; mediaan (uitersten)	17 *(0-87)*	*17 (0-152)*	*2 (0-13)*	*28 (14-100)*	*31 (15-152)*

Symptoomvrije dagen

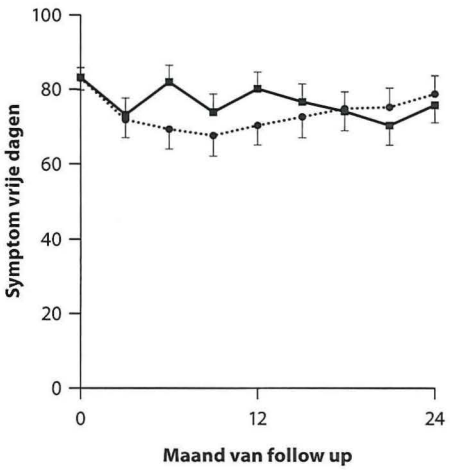
Het gemiddelde percentage symptoomvrije dagen nam in beide armen in gelijke mate toe: van 50 (SEM: 4) tot 71% (SEM: 3) in de referentiearm en van 47 (SEM: 3) tot 69% (SEM: 3) in de BHR-arm (beide $p < 0{,}001$; figuur 2). Het gecorrigeerde verschil tussen de 2 armen in de laatste 3 maanden was -1,1% (95%-BI: -10,1-7,9; $p = 0{,}84$; BHR- minus referentiestrategie). Ook in de subgroepen was het verschil tussen de beide behandelstrategieën niet significant.

***Figuur 2** - Gemiddeld percentage symptoomvrije dagen als functie van de tijd voor Referentiestrategie (■) en bronchiale hyperreactiviteit (BHR)-strategie (•). Foutenbalken geven de standaardfout van het gemiddelde (SEM) bij elk bezoek aan. Het gecorrigeerde verschil in het gemiddelde percentage symptoomvrije dagen tijdens de laatste 3 maanden was niet verschillend tussen de strategiearmen (correctie vond plaats voor het verschil in percentage symptoomvrije dagen tussen de strategiearmen tijdens de inloopperiode). (A) Totale onderzoekspopulatie (n = 206); (B) BHR-subgroep (n = 91): kinderen met BHR en weinig symptomen tijdens inloopperiode van de studie; (C) S-subgroep (n = 46): kinderen met duidelijke symptomen, zonder BHR tijdens inloopperiode; (D) S+BHR-subgroep (n = 69): kinderen met duidelijke symptomen en BHR tijdens inloopperiode.*

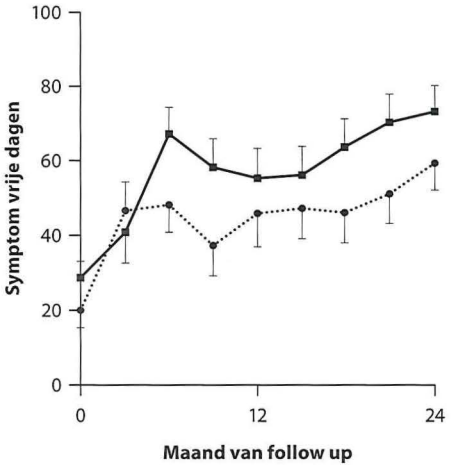
Figuur 2A



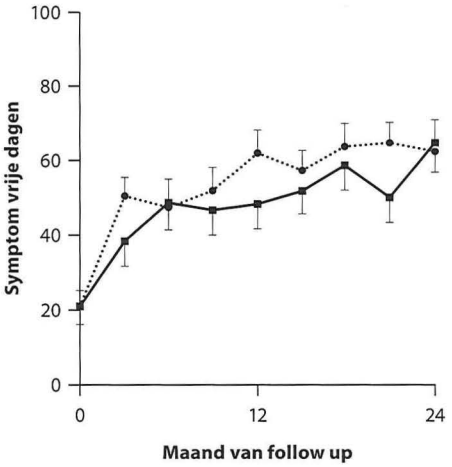
Figuur 2B



Figuur 2C



Figuur 2D

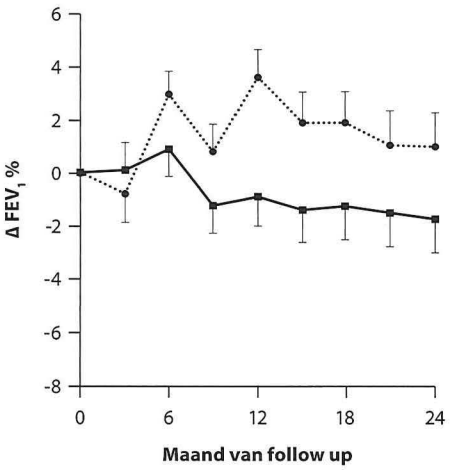


Longfunctie en BHR

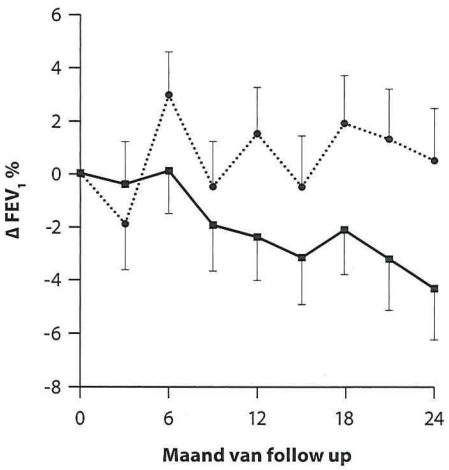
Na 2 jaar was in de BHR-strategiearm het gemiddelde FEV_1 (% voorspelde waarde) 2,3% hoger dan in de referentiearm (95%-BI: 0,05–4,6; p = 0,046; figuur 3A). Kinderen met BHR en goede symptoomcontrole (BHR-subgroep) behandeld volgens de BHR-strategiearm hadden een hoger gemiddeld FEV_1 dan kinderen in de referentiearm (p = 0,024). Het verschil in FEV_1 na 2 jaar was 6,0% (95%-BI: 1,2–10,8; p = 0,017; figuur 3B). Dit verschil werd verklaard door een geleidelijke afname van het FEV_1 in de referentiearm (p = 0,027), terwijl in de BHR-strategiearm het FEV_1 gelijk bleef (p = 0,23).

***Figuur 3** - Verandering (Δ) van het geforceerde expiratoire 1-secondevolume (FEV_1; in % voorspelde waarde) ten opzichte van de beginmeting als functie van de tijd, voor de referentiestrategie (■) en bronchiale-hyperreactiviteit (BHR) strategie (•). Foutenbalken geven de standaardfout van het gemiddelde (SEM) bij elk bezoek aan. (A) Totale onderzoekspopulatie (n = 206). Tijdens de behandelperiode was het gemiddelde verschil in verandering vanaf begin van de studie in FEV_1 2,3% van de voorspelde waarde (p = 0,046); (B) BHR-subgroep (n = 91): kinderen met BHR en weinig symptomen tijdens inloopperiode van de studie. Na 2 jaar was het gecorrigeerde verschil in verandering vanaf het uitgangs-FEV_1 tussen de 2 behandelstrategieën 6,0% van de voorspelde waarde (p = 0,017).*

Figuur 3A



Figuur 3B



In de subgroepen S en S+BHR werd geen significant verschil in FEV_1 tussen de behandelstrategieën gevonden. Kinderen in de BHR-subgroep die werden behandeld volgens de BHR-strategie hadden na 2 jaar 1,6% hogere FEV_1/FVC-waarden (95%-BI: 0,3–2,9; p = 0,015). De andere 2 subgroepen vertoonden geen significant verschil. De PD_{20} verbeterde met beide behandelstrategieën in gelijke mate.

Astma-aanvallen

17 van de 104 (16,3%) kinderen in de referentiearm en 16 van de 102 (15,7%) in de BHR-strategiearm hadden tenminste 1 astma-aanval ($p = 0,69$). In het jaar vóór het onderzoek was dit respectievelijk 12 (11,5%) en 25 (24,5%).

Bijwerkingen

Aan het einde van het onderzoek werd geen significant verschil in groeisnelheid waargenomen bij de kinderen in de 2 behandelstrategieën (aangepast verschil ten opzichte van de aanvangsmeting: -0,1 SD-score; 95%-BI: -0,2–0,04; $p = 0,18$). In de BHR-subgroep werd echter aan het einde van het onderzoek een significant lengteverschil waargenomen tussen de kinderen met verschillende behandelstrategieën (aangepast verschil: -0,4 SD-score; 95%-BI: -0,6–-0,2; $p < 0,001$). Gegevens over de groei waren voor niemand reden om deelname aan het onderzoek te beëindigen.

BESCHOUWING

Bij kinderen met atopisch astma gaf behandeling mede op geleide van BHR vergeleken met behandeling uitsluitend op geleide van symptomen geen verdere toename van het aantal symptoomvrije dagen. Behandeling mede op geleide van BHR verhinderde wel de afname van longfunctie op lange termijn, vooral bij een grote subgroep kinderen met BHR en weinig klachten. Behandeling van astma uitsluitend op geleide van symptomen leidde in deze subgroep na 2 jaar tot longfunctieverlies. Het feit dat 44% van onze onderzoekspopulatie in eerste instantie lage symptoomscores had ondanks BHR suggereert dat dit fenotype astma veel voorkomt in onze secundaire en tertiaire zorg. Dit is het eerste onderzoek dat aanwijzingen geeft dat een behandeling die is aangepast aan het fenotype leidt tot een beter resultaat op een middellange termijn van 2 jaar.

Ontsteking gaat gepaard met structurele veranderingen in de luchtwegen en een van de belangrijkste functionele gevolgen van luchtwegontsteking en -remodellering is BHR.[13] Langdurige behandeling met ICS geeft een geleidelijke verbetering van BHR.[21] Dit kan een belangrijk therapeutisch doel zijn, omdat persisterende BHR een risicofactor is voor sneller verlies van longfunctie op lange termijn.[1] Ook is BHR gecorreleerd met persisteren van astma op volwassen leeftijd. Bij jongvolwassenen met astma wordt op 18-jarige leeftijd al een verlies van longfunctie gerapporteerd ten opzichte van gezonde personen.[2] Late introductie van ICS of ontoereikende dosering kan een dergelijke longfunctiestoornis bevorderen.[22]

Waarom bleef de longfunctie met de BHR-strategie beter? Gegevens over de effecten van ICS-behandeling op luchtwegremodellering zijn tegenstrijdig.[14,23,24] BHR is een risicofactor voor snel longfunctieverlies bij astma en de afname van de longfunctie met de leeftijd bij astmapatiënten correleert met de ernst van BHR.[1,21,25] BHR is omgekeerd gerelateerd aan de luchtwegdiameter.[26] Het voorkómen van verlies van longfunctie door de BHR-strategie kan dus het gevolg zijn van het effect van ICS op luchtwegremodellering.

Met beide strategieën nam het aantal symptoomvrije dagen toe. Mogelijke verklaringen zijn een medicatie-effect, een verhoogde therapietrouw of een spontane verbetering van de ziekte. Een behandeleffect van de ICS en/of de langwerkende luchtwegverwijders lijkt plausibel. Deze laatste categorie medicijnen werden volgens de GINA-richtlijnen gebruikt en kunnen mede van invloed zijn geweest op de ernst van de symptomen.

We vonden een kleine, maar significante vermindering van de groeisnelheid in de BHR-subgroep. Deze subgroep had gemiddeld de hoogste dosis ICS. Uit onderzoek naar de groei van astmatische kinderen blijkt dat de groeisnelheid vaak afneemt tijdens de eerste maanden van ICS-behandeling, maar dat dit geen invloed heeft op de eindlengte.[27]

Wat zijn de klinische implicaties van ons onderzoek? Astmatische kinderen met evidente BHR, ondanks goede symptoomcontrole, blijken een risico te lopen op sneller longfunctieverlies wanneer behandeling uitsluitend gebaseerd wordt op de ernst van hun klachten. Wij laten zien dat behandeling op geleide van BHR bij dergelijke patiënten na 2 jaar resulteert in een hogere FEV_1. Omdat provocatietests met methacholine tijdrovend en ongebruikelijk zijn in de kindergeneeskundige praktijk is het routinematig meten van de BHR bij alle kinderen met astma niet raadzaam. Het lijkt echter wel zinvol om kinderen met weinig symptomen op BHR te testen, om degenen met kans op relatief snelle afname van de longfunctie te identificeren.

CONCLUSIE

Wij concluderen dat astmabehandeling op geleide van de BHR, vergeleken met een behandeling op geleide van symptomen, geen verdere verbetering van het aantal symptoomvrije dagen geeft, maar longfunctieverlies op de lange termijn voorkómt. Dit laatste geldt vooral voor een grote subgroep kinderen met evidente BHR ondanks weinig klachten. Dit zou kunnen betekenen dat individuele, fenotypespecifieke behandeling van astma op de kinderleeftijd de prognose van deze aandoeningen op volwassen leeftijd zal verbeteren.

LITERATUUR

1 Grol MH, Gerritsen J, Vonk JM, Schouten JP, Koëter GH, Rijcken B, et al. Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. A 30-year follow-up study. Am J Respir Crit Care Med. 1999;160:1830-7.

2 James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, et al. Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. Am J Respir Crit Care Med. 2005;171:109-14.

3 Rabe KF, Adachi M, Lai CK, Soriano JB, Vermeire PA, Weiss KB, et al. Worldwide severity and control of asthma in children and adults: the global asthma Insights and Reality surveys. J Allergy Clin Immunol. 2004;114:40-7.

4 Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids. New developments. Am J Respir Crit Care Med. 1998;157:S1-53.

5 National Heart, Lung, and Blood Institute. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention NHLBI/WHO Workshop. NIH Publication No. 95-3659. Bethesda: National Institutes of Health; 2004.

6 Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, et al. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. Am Rev Respir Dis. 1992;145:669-74.

7 Sont JK, van Krieken JH, Evertse CE, Hooijer R, Willems LNA, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Thorax. 1996;51:496-502.

8 Van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med. 2001;164:2107-13.

9 Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet. 2002;360:1715-21.

10 Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemiere C, Pizzichini E, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. Eur Respir J. 2006;27:483-94.

11 Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. N Engl J Med. 2005;352:2163-73.

12 Pijnenburg MW, Bakker EM, Hop WC, de Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. Am J Respir Crit Care Med. 2005;172:831-6.

13 Boulet LP, Chakir J, Dubé J, Laprise C, Boutet M, Laviolette M. Airway inflammation and structural changes in airway hyper-responsiveness and asthma: an overview. Can Respir J. 1998;5:16-21.

14 Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med. 1999;159:1043-51.

15 Bijl-Hofland ID, Cloosterman SGM, Folgering HT, Akkermans RP, van Schayck CP. Relation of the perception of airway obstruction to the severity of asthma. Thorax. 1999;54:15-9.

16 Koh YI, Choi IS, Lim H. Airway responsiveness as a direct factor contributing to the dyspnoea perception in asthma. Respir Med. 2001;95:464-70.

17 Verberne AA, Frost C, Roorda RJ, van der Laag H, Kerrebijn KF. One year treatment with salmeterol compared with beclomethasone in children with asthma. The Dutch Paediatric Asthma Study Group. Am J Respir Crit Care Med. 1997;156:688-95.

19 Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J. 1993;6:5-40.

20 Birnie D, Schwartzenberg GW thoe, Hop WC, Essen-Zandvliet EE van, 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;45:199-202.

21 Pauwels RA, Löfdahl C G, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. N Engl J Med. 1997;337:1405-11.

22 Woolcock AJ, Yan K, Salome CM. Effect of therapy on bronchial hyperresponsiveness in the long-term management of asthma. Clin Allergy. 1988;18:165-76.

23 Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med. 2003;349:1414-22.

24 Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. Thorax. 2002;57:309-16.

25 Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson SA. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma. A quantitative light and electron microscopic study. Am Rev Respir Dis. 1992;145:890-9.

26 Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, et al. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV1/vital capacity ratio: a longitudinal population study from childhood to adulthood. Am J Respir Crit Care Med. 2002;165:1480-8.

27 Xuan W, Peat JK, Toelle BG, Marks GB, Berry G, Woolcock AJ. Lung function growth and its relation to airway hyperresponsiveness and recent wheeze. Results from a longitudinal population study. Am J Respir Crit Care Med. 2000;161:1820-4.

28 Brand PL. Inhaled corticosteroids reduce growth. Or do they? Eur Respir J 2001;17:287-94.