Clinical Control and Histopathologic Outcome of Asthma when Using Airway Hyperresponsiveness as an Additional Guide to Long-Term Treatment

JACOB K. SONT, LUUK N. A. WILLEMS, ELISABETH H. BEL, J. HAN J. M. van KRIEKEN, JAN P. VANDENBROUCKE, PETER J. STERK, and the AMPUL Study Group

Departments of Pulmonology, Clinical Epidemiology, and Pathology, Leiden University Medical Center; and Asthma Management Project University Leiden (AMPUL) Study Group, Leiden, The Netherlands

According to international guidelines, the level and adjustment of antiinflammatory treatment for asthma are based solely on symptoms and lung function. We investigated whether a treatment strategy aimed at reducing airway hyperresponsiveness (AHR strategy) in addition to the recommendations in the existing guidelines (reference strategy) led to: (*1*) more effective control of asthma; and (*2*) greater improvement of chronic airways inflammation. To accomplish this, we conducted a randomized, prospective, parallel trial involving 75 adults with mild to moderate asthma who visited a clinic every 3 mo for 2 yr. At each visit, FEV_1 and AHR to methacholine were assessed, and subjects kept diaries of symptoms, β_2-agonist use, and peak expiratory flow (PEF). Medication with corticosteroids (four levels) was adjusted according to a stepwise approach (reference strategy), to which four severity classes of AHR were added (AHR strategy). At entry and after 2 yr, bronchial biopsies were obtained by fiberoptic bronchoscopy. Patients treated according to the AHR strategy had a 1.8-fold lower rate of mild exacerbations than did patients in the reference strategy group (0.23 and 0.43 exacerbation/yr/patient, respectively). FEV_1 also improved to a significantly greater extent in the AHR strategy group ($p \leq 0.05$). In bronchial biopsies this was accompanied by a greater reduction in thickness of the subepithelial reticular layer in the AHR strategy group than in the reference strategy group (mean difference [95% confidence interval (CI): 1.7 μm (0.2 to 3.1) μm]). The changes in AHR in both strategy groups were correlated with eosinophil counts in the biopsies ($r = -0.48$, $p = 0.003$). We conclude that reducing AHR in conjunction with optimizing symptoms and lung function leads to more effective control of asthma while alleviating chronic airways inflammation. This implies a role for the monitoring of AHR or other surrogate markers of inflammation in the long-term management of asthma. Sont JK, Willems LNA, Bel EH, van Krieken JHJM, Vandenbroucke JP, Sterk PJ, and the AMPUL Study Group. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment.

AM J RESPIR CRIT CARE MED 1999;159:1043–1051.

Asthma is a chronic inflammatory disorder of the airways characterized by recurrent episodes of symptoms of wheezing and chest tightness that are associated with variable airways obstruction (1). These features can be provoked by exposure to bronchoconstrictive stimuli in the laboratory, thereby demonstrating airway hyperresponsiveness (AHR) (1). The accompanying airways inflammation is characterized by a specific infiltrate of mast cells, lymphocytes, and eosinophils in the bronchial epithelium and lamina propria (2), and by thickening of the subepithelial reticular layer (3), even in patients with mild and newly diagnosed asthma (4). Hence, the disease state can be assessed in different ways: according to severity of symptoms, level of airflow limitation, degree of AHR, and extent of airway pathology.

Present guidelines on asthma management adhere to the concept that the major goal of treatment is to reverse or prevent airways inflammation with so-called controller medication (1). Thus, inhaled corticosteroids are introduced into the treatment of asthma when inhaled bronchodilators alone are inadequate to control signs and symptoms of the disease (1). In addition, it is recommended that objective measures of lung function (such as peak flow) be added to monitor the degree of airways obstruction. Hence, according to the current step-

(*Received in original form June 10, 1998 and in revised form September 15, 1998*)

C. R. Apap (St. Antoniushove Hospital, Leidschendam); A. C. Roldaan, K. W. van Kralingen (Langeland Hospital, Zoetermeer); H. C. J. van Klink (Diaconessenhuis Hospital, Leiden), C. E. Evertse, J. K. Sont, L. N. A. Willems, E. H. Bel, P. J. Sterk (Department of Pulmonology, Leiden University Medical Center (LUMC), Leiden), J. K. Sont, J. P. Vandenbroucke (Department of Clinical Epidemiology, LUMC); P. Neeskens, W. A. A. M. van Schadewijk, R. Hooijer, G. Cramer, J. H. J. M. van Krieken (Department of Pathology, LUMC) and K. Dijkstra, N. Dijkstra, and J. C. Kluin-Nelemans (Department of Hematology, LUMC).

Supported by Grant 92.45 of The Netherlands Asthma Foundation.

Correspondence and requests for reprints should be addressed to Prof. P. J. Sterk, Lung Function Laboratory, C2-P, Department of Pulmonology, Leiden University Medical Center, P.O. Box 9600, NL-2300 RC Leiden, The Netherlands. E-mail: psterk@pulmonology.azl.nl

Am J Respir Crit Care Med Vol 159. pp 1043–1051, 1999
Internet address: www.atsjournals.org

wise strategy, the level and adjustment of antiinflammatory treatment for asthma is guided solely by symptoms and lung function (1).

However, in many patients whose disease is considered to be clinically controlled, it appears that AHR and airways inflammation persist (5, 6). The concept that the chronicity of such abnormalities may lead to airways remodeling, thereby worsening the long-term outcome of asthma (7, 8), has prompted many efforts to identify (noninvasive) markers of inflammation for improved monitoring of patients with the disease (9, 10).

Airways inflammation is associated with AHR to bronchoconstrictive stimuli in asthma (6, 11). Hence, it can be postulated that AHR can serve as a guide to asthma therapy (12, 13). This might benefit the long-term outcome of asthma as formulated in the international guidelines for its management, by preventing airway remodeling and the development of irreversible airway obstruction (8, 14). Alternatively, it can be argued that such a treatment strategy is unwarranted, since the relationship between AHR and airway inflammation is not a consistent one in asthma (15). We therefore addressed, in the present study, the question of whether the treatment of asthma should be directed toward reducing AHR in addition to optimizing symptoms and lung function.

In a 2-yr prospective, randomized, single-blind parallel trial, we adjusted controller medication with inhaled corticosteroids for asthma according to a stepwise approach, corresponding to the international guidelines (1), based either on symptoms and lung function alone (reference strategy) or additionally on the degree of AHR to methacholine (AHR strategy). We hypothesized that a management strategy aimed at reducing AHR in addition to improving symptoms and lung function would lead to more effective control of asthma, fewer exacerbations, less variable airflow limitation, and more effective reduction of airways inflammation and remodeling. To that end, we assessed the effect of the two treatment strategies on these outcomes in an overall analysis by strategy arm, and in a separate analysis by indication for a higher steroid requirement according to the severity of AHR.

TABLE 1

CHARACTERISTICS OF 75 PATIENTS WITH ATOPIC ASTHMA RANDOMIZED TO THE REFERENCE OR AHR STRATEGY

Characteristic	Reference Strategy (*n* = *41*)	AHR Strategy (*n* = *34*)
Age, SD	28.2 (1.3)	31.5 (1.7)
Sex, M/F	20/21	17/17
Height, m, mean (SD)	1.73 (1.42)	1.72 (1.75)
FEV_1, % pred, mean (SD)		
Prebronchodilator	94.6 (2.1)	89.0 (2.6)
Postbronchodilator	103.6 (1.8)	101.1 (2.1)
Methacholine PC_{20}, mg/ml		
Geometric mean (SD)*	0.82 (0.36)	0.47 (0.36)
PEF variability		
Geometric mean (-fold SD)	7.3 (1.7)	9.5 (1.9)
Newly diagnosed, no. (%)	13 (32)	9 (26)
Regular inhaled steroids, no. (%)	28 (68)	25 (74)
Undergoing bronchoscopy, no. at entry (end)	29 (26)	26 (23)
Baseline treatment level, no. (%)†		
Step 4, high-dose steroids	1 (2)	5 (14)
Step 3, intermediate-dose steroids	9 (22)	6 (18)
Step 2, low-dose steroids	22 (54)	15 (44)
Step 1, no steroids	9 (22)	8 (24)

Definition of abbreviation: AHR = airway hyperresponsiveness.

* SD in doubling doses.

† Baseline treatment level was based on the reference strategy algorithm. None of these baseline characteristics differed significantly in the two strategy groups.

METHODS

Patients

We recruited 75 patients who were visiting a chest physician for their asthma at one of the outpatient clinics of four hospitals in the Leiden area, and who met the inclusion criteria for our study (Table 1). All of the patients were nonsmokers at the time of recruitment (> 1 yr; < 5 pack-yr), and were atopic, between 18 and 50 yr of age, and had had a history of episodic chest tightness and wheezing in the previous year. Atopy was assessed through a positive skin-prick test (> 3 mm wheal) to one or more common airborne allergen extracts (Soluprick; ALK, Copenhagen, Denmark). Prebronchodilator FEV_1 was more than 50% predicted (16) and > 1.5 L, whereas postbronchodilator FEV_1 was within the normal range (> 80% predicted). At the time of patients' entry into the study, AHR was established through a 20% decrease in FEV_1 in response to a provocative concentration of inhaled methacholine (PC_{20}) of < 8 mg/ml. Subjects were eligible when they had used no other medication than regular inhaled steroids and/or β-agonists as needed for their asthma during the 6 mo before entry. All subjects gave their written informed consent, and the study was approved by the local medical ethics committee.

Design: Management Strategies

The study was a prospective, randomized, single-blind, parallel trial with a 2-yr follow-up. It compared a treatment strategy aimed at reducing AHR (AHR strategy) in addition to parameters recommended in existing guidelines for the management of asthma with a strategy that was similar to the existing guidelines (reference strategy). The patients visited their chest physician at one of the participating outpatient clinics every 3 mo during the 2-yr study period. Starting 2 wk before each visit, the patients kept a diary with symptom scores, morning and evening peak expiratory flow (PEF) readings, and medication usage. At each visit, spirometry was performed and a methacholine inhalation challenge test was conducted. At the beginning of the study and after 2 yr of follow-up, pre- and postbronchodilator FEV_1 were determined before and after inhalation of 400 μg salbutamol by metered dose inhaler. Additionally, all patients were asked to undergo fiberoptic bronchoscopy at entry into and at the end of the study.

Following the measurements made at each study visit, controller medication was adjusted according to a stepwise approach. Implicitly, the treating physician could not be blinded with regard to the two arms of the study. Therefore, the treatment and its adjustment at each 3 mo visit were standardized through a computerized algorithm. In both the reference strategy and the AHR strategy, this was guided by four severity classes of four different clinical markers: symptoms, bronchodilator usage, diurnal variability in PEF, and FEV_1 level (Table 2). The severity classes originated from published international guidelines on the diagnosis and treatment or asthma (1, 8, 17). The required treatment level (four steps) for the 3 mo subsequent to each visit was guided by the highest among the severity classes according to existing recommendations (1). In the AHR strategy, four severity classes of PC_{20} were added, and treatment was further adjusted if the PC_{20} class exceeded the classes found for the other four markers (Table 2).

The four steps in the treatment with controller medication were: (*1*) no requirement for corticosteroids; (*2*) low-dose inhaled steroids (2 × 200 μg budesonide by dry-powder inhaler [Turbuhaler; Astra Draco, Lund, Sweden] or beclomethasone dipropionate by metered dose inhaler [Glaxo-Wellcome, Middlesex, UK]); (*3*) intermediate-dose inhaled steroids (2 × 400 μg); (*4*) high-dose inhaled steroids (2 × 800 μg/d) plus a short course of oral prednisone (30 mg/d for 2 d, with subsequent doses decreasing by 5 mg every 2 d) (13). Symptoms were additionally controlled by use of short-acting β_2-agonists as needed (1).

Clinical Measurements

Diary card symptoms, medication usage, and morning/evening PEF were recorded as previously described (6). PEF variability was calculated from the highest minus the lowest daily value in PEF divided by the highest value over a 14-d period. FEV_1 was measured with stan-

TABLE 2

SEVERITY CLASSES OF SYMPTOMS, BRONCHODILATOR USAGE, DIURNAL VARIABILITY IN PEF, FEV_1, AND AIRWAY HYPERRESPONSIVENESS WITH CORRESPONDING TREATMENT STEPS

	Both Strategies				AHR Strategy Only
Treatment Step	Symptoms > 3 d/2 wk	Bronchodilator Use	PEF Variability (%)	FEV_1 (% pred)	Airway Hyperresponsiveness: Methacholine PC_{20} (mg/ml)
4	Disturbed sleep/early wakeup/limited physical activities	⩾ 4 hourly	> 50	< 50	< 0.25
3	Nighttime symptoms/early wakeup/affect activities	⩾ 6 hourly	30–50	50–60	0.25–1.0
2	Mild nighttime/morning symptoms/may affect activities	1–4 ×/d	20–30	60–70	1.0–4.0
1	< 3 d/2 wk	< Daily	< 20	> 70	> 4.0

Subjects recorded symptoms of nighttime asthma, morning tightness, daytime asthma, and daytime cough. Each item could range from 0 to 4 on each day. The highest class among these 4 items, on at least 3 d during a 14-d period, determined the symptom severity. Bronchodilator usage was obtained as the number of total puffs of salbutamol or ipratropium bromide during a period of 14 d registered on the diary cards, and was expressed per week. PEF values were obtained from twice-daily measurements, on waking and before bedtime, and from additional measurements when bronchodilator had been used. Diurnal variability in PEF was expressed as the mean value for 14 d calculated from the highest minus the lowest daily value, divided by the highest daily value (17).

dardized spirometry (16). AHR was measured through 2-min methacholine challenge tests at tidal breathing, and was expressed as PC_{20} for FEV_1 (6, 18). Briefly, methacholine was used in doubling concentrations (0.03 to 256 mg/ml) delivered from a DeVilbiss 646 nebulizer (DeVilbiss, Somerset, PA) carrying oxygen (output 130 mg/min) and connected to the central chamber of an inspiratory and expiratory valve box with an expiratory aerosol filter (Pall Ultipor BB50T). Volume was measured with a dry rolling-seal spirometer (Morgan Spiroflow, Rainham/Gillingham, UK). The challenge test was discontinued if FEV_1 fell by more than 20% from its baseline value or fell below 0.75 L; if the patient felt uncomfortable; or if the highest concentration of methacholine had been given. Spirometric and AHR results were recorded by the same lung function technician, and were stored on-line in a laptop computer together with the patients' diary card readings.

Bronchoscopy

Fiberoptic bronchoscopy was done by an experienced investigator (L.N.A.W.), using a standardized protocol according to previous recommendations (19, 20). Each procedure involved detailed explanation, premedication, local anesthesia, bronchoscopy, and sampling according to a recently published protocol (6). Five bronchial biopsy specimens were taken (for electron microscopy [EM] and light microscopy [LM] from right lower lobe subsegments, the middle lobe (LM) and the main carina (EM and LM), using a pair of cup forceps (Olympus FB-21C, Tokyo, Japan).

Processing and Analysis of Endobronchial Biopsies: EM

Two biopsy samples were immediately fixed (1 to 2 h, 20° C) in Trump's fixative (0.03 M Na-cacodylate buffer, 4% formalin, 1% glutaraldehyde) and subsequently stored in 0.14 M Na-cacodylate buffer pending further processing. Samples were postfixed (1.5 to 2 h, 20° C) in 1% osmium tetroxide with 1% K_4FeCN_6 and in 1.5% uranyl acetate (1.5 to 2 h, 20° C) before dehydration through dimethoxypropane and acetone, and were embedded in Spurr's epoxy resin. One-micrometer-thick sections, stained with Richardson's methylene blue, were used to orient the specimens for assessment of the surface epithelium. The area with the best preserved and oriented surface epithelium was trimmed for ultrathin sections and further processed for quantification by EM.

Quantification of the reticular-layer thickness was done according to a validated method by dividing the area by the length of the reticular layer in two to five well oriented electron micrographs (3) (×5,700, 35 × 42 μm), using a video interactive display analysis system (VIDAS II; Kontron Electronik GmbH, Munich, Germany).

Processing and Analysis of Endobronchial Biopsies: LM

Biopsy samples were immediately embedded in ornithyl carbamyltransferase (OCT) medium (Miles Inc., Diagnostics Division, Elkhart, IN) and snap-frozen in isopentane cooled with iced CO_2. Samples were stored at −70° C pending further processing. Six-micrometer-thick cryostat sections were air dried for 1 h and fixed in a mixture of acetone:methanol (1:1, vol/vol) for 2 min. Besides hematoxylin-eosin (H&E) staining, immunohistochemistry was performed with an indirect immunoperoxidase technique, using monoclonal antibodies directed against the secreted form of eosinophil cationic protein (EG2) (Pharmacia, Uppsala, Sweden); mast cell tryptase (AA1) (DAKO, Glostrup, Denmark); CD45, CD3 (leu4), CD4 (leu3), CD8 (leu2) (all Becton Dickinson, Mountain View, CA); and CD45RO (UCHL1) (DAKO).

All biopsy specimens were coded and sections were blindly examined at a magnification of ×200 by one investigator (J.K.S.) by means of a validated method, using a video interactive display analysis system (6, 21). Two areas of 0.123 mm^2 each were randomly chosen and presented on a video screen. Subsequently, the area of lamina propria was determined by delineating the widest possible 125-μm-deep zone beneath the epithelial basement membrane (at least 20,000 μm^2), excluding damaged tissue and airway smooth muscle. Positively stained cells were counted within this area and expressed as the number of cells/0.1 mm^2.

Outcome Variables

Five main outcome variables were examined during the 2-yr follow-up period: the rate of mild exacerbations of asthma, changes in FEV_1 from baseline (before and after bronchodilation), the variability in prebronchodilator FEV_1, changes in reticular layer thickness, and the number of inflammatory cells in the lamina propria. Severe exacerbations requiring treatment with oral prednisone as judged by the chest physician (doses decreasing by 5 mg every 2 d, as follows: 30, 30, 25, 25, 20, 20, 15, 15, 10, 10, 5, 5 mg/d) did not occur frequently enough to be used as an outcome variable. Hence, mild exacerbations of asthma were defined as an increase of at least 3 points in the total asthma score obtained as the sum of severity classes of the following features: symptoms, bronchodilator usage, PEF variability, and FEV_1 (Table 2) (8). Multiple occurrences of an exacerbation per patient were included in the analysis. Changes in prebronchodilator FEV_1 during the trial were expressed as the area under the changes-versus-time curve (22). Variability in airways obstruction was assessed from the individual standard deviation (SD) of prebronchodilator FEV_1 over the final 18 mo of the study.

Reticular layer thickness was averaged for two to five electron micrographs per location and for the two locations, and its assessment showed good intraobserver reproducibility (intraclass correlation coefficient: 0.9). Similarly, numbers of positively stained cells were averaged over the three localizations. Cell counts were log-transformed before analysis to overcome heteroscedasticity (i.e., increasing variability with higher cell numbers). Changes in cell number could then be expressed as fold increase/decrease (21).

Statistical Analysis

A chi-square test was used to assess whether baseline treatment levels were significantly different for the two management strategies. Unpaired and paired Student's *t* tests were applied to test the changes in outcome variables within and between the two strategies. The incidence of mild exacerbations of asthma per patient per year (person-

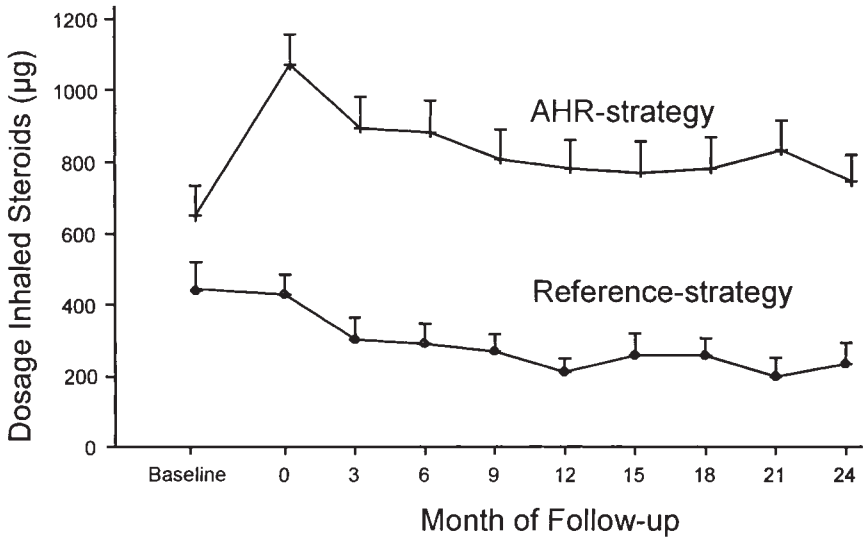

Figure 1. Actual daily doses of inhaled steroids (µg; mean ± SEM) according to the AHR strategy and the reference strategy. The median difference in treatment with inhaled steroids was ± 400 µg during the 2-yr follow-up. Treatment requirement decreased with both strategies. However, the decrease with the AHR strategy was somewhat greater than with the reference strategy.

year) during follow-up was calculated. The cumulative incidence of exacerbations was determined and survival curves were constructed by the Kaplan–Meier method, using first exacerbations of asthma only. A possible difference in exacerbation rate was examined through a variant of the Cox regression that allows the use of multiple (correlated) occurrences of exacerbations per patient by robust variance estimates (23). First, differences in exacerbation rate were assessed by strategy arm (AHR strategy versus reference strategy). Second, analysis by indication was done by separating out patients in the reference arm who had a positive indication through AHR but were not treated accordingly and comparing them with patients in the AHR arm who had a positive indication through AHR and who were treated accordingly and comparing both of these patient subgroups with patients without a positive indication as a result of AHR. Whether the level of AHR or changes in this level reflect the degree of airways inflammation or changes in such inflammation was assessed by correlation analysis. All statistical analyses were done with the statistical software package STATA 5.0 (StataCorp, College Station, TX). The 95% confidence intervals (95% CI) are given whenever appropriate. Values of $p < 0.05$ were considered significant.

RESULTS

Of the 75 patients who completed the baseline period of the study, 55 agreed to undergo fiberoptic bronchoscopy (Table 1). Twenty-three patients had newly detected asthma and were not using any corticosteroids at entry into the study, whereas the other 52 patients had been taking regular inhaled corticosteroids for an average of 20 ± 27 months (mean ±

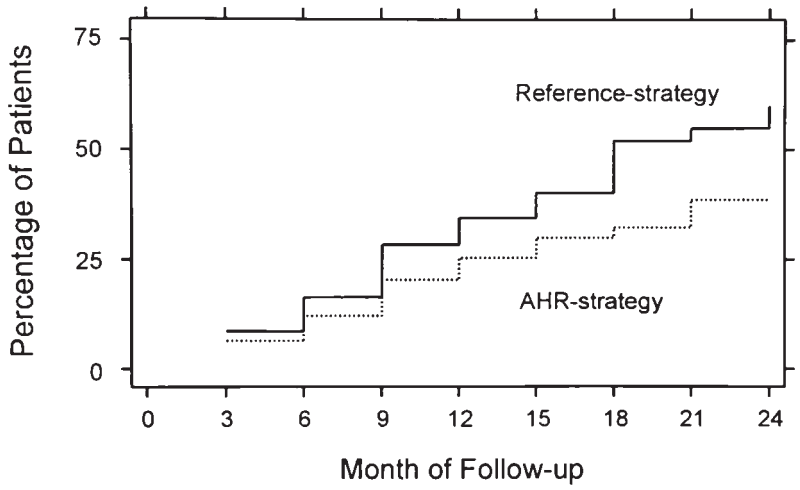

Figure 2. Cumulative incidence of first mild asthma exacerbations by treatment strategy. The occurrence of a mild exacerbation at the 3-monthly visits was defined as at least a 3-point increase in a total asthma score that was obtained by the sum of the severity classes of symptoms, bronchodilator usage, diurnal variability in PEF, and FEV_1 (Table 1). There was a 1.8-fold decrease in exacerbation rate in the AHR strategy as compared with the reference strategy group ($p = 0.03$).

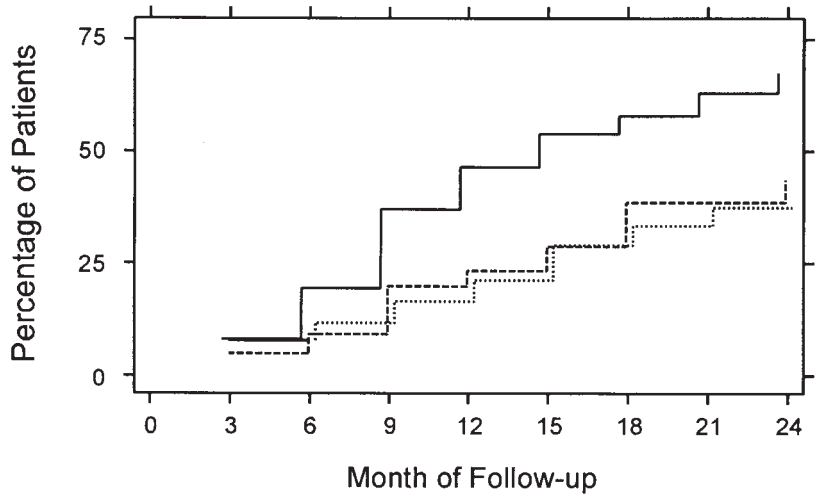

Figure 3. Cumulative incidence of first mild asthma exacerbations according to indication by AHR. Patients who did require a higher level of treatment with inhaled steroids according to indication by AHR (relatively severe hyperresponsiveness), but who were not treated accordingly (*continuous line*), had a 2.1-fold higher exacerbation rate than patients who did not require a higher level of treatment (relatively mild hyperresponsiveness) (*dashed line*) ($p = 0.04$). On the other hand, patients who did require a higher level of treatment with inhaled steroids according to indication by AHR, and who were treated accordingly (*dotted line*), had a similar prognosis as patients who did not require a higher level of treatment according to indication by AHR (*dashed line*) (hazard ratio: 0.92; $p = 0.84$).

SD). A total of 67 patients completed the 2-yr follow-up period (reference strategy group: $n = 35$, six dropouts; AHR strategy group: $n = 32$, two dropouts), in 49 of whom an endpoint bronchoscopy was performed. None of the characteristics of patients differed significantly between the two strategy groups (Table 1).

At baseline, the two patient groups did not differ with respect to treatment step according to the reference strategy (chi-squared = 4.03, $p = 0.26$). At baseline and at all subsequent visits, a step-up in treatment was indicated and applied in 66% of all visits in the AHR strategy group, whereas it was indicated by AHR but not applied in 61% of all visits in the reference strategy group. Subsequently, during follow-up, treatment Steps 1, 2, 3, and 4 were reached in 46%, 37%, 13%, and 4%, respectively, of all visits in the reference strategy group, and in 7%, 35%, 38%, and 20%, respectively, of all visits in the AHR strategy group. This led to a stepping up toward low and intermediate doses of inhaled steroids in 47% of the patient visits and toward high doses in only 16%. The average difference between the two strategies in steroid dose

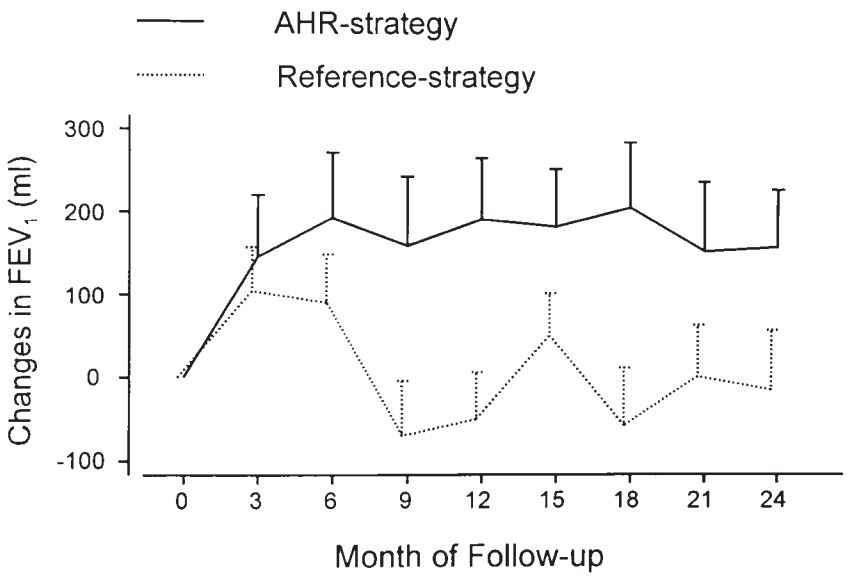

Figure 4. Changes in mean prebronchodilator FEV_1 by month of follow-up for the AHR- and reference strategy groups. *Error bars* indicate the SEM at each visit. The area under the time–response curve was greater in the AHR strategy group than in the reference strategy group ($p = 0.05$).

TABLE 3

CHANGES IN CLINICAL OUTCOME VARIABLES FROM BASELINE TO 2 yr OF FOLLOW-UP

Outcome Variable	Reference Strategy Mean (SD)	AHR Strategy Mean (SD)	Mean Difference	95% CI	p Value
Prebronchodilator FEV_1					
Baseline, L	3.54 (0.76)	3.26 (0.94)	0.28	−0.12–0.68	0.16
Change, ml/yr	−7 (36)	78 (34)§	86	−15–186	0.09
AUC, L · mo‡	0.19 (1.17)	4.48 (1.51)§	−4.3	−8.07–−0.52	0.02
Individual SD, L	0.19 (0.11)	0.14 (0.81)	0.05	0.0–0.09	0.05
Postbronchodilator FEV_1					
Baseline, L	3.97 (0.79)	3.79 (0.96)	0.18	−0.23–0.58	0.38
Change, ml/yr	−75 (15)§	−75 (16)§	0	−78–77	0.99
Methacholine PC_{20}					
Baseline, mg/ml*	0.82 (2.1)	0.47 (2.0)	0.13	−0.23–0.58	0.38
Change, doubling dose	0.46 (2.4)	1.1 (1.5)‖	−0.64	−1.62–0.33	0.19

* Geometric mean (SD in doubling dose).
‡ AUC = area under the time-response curve.
§ $p < 0.05$.
‖ $p < 0.01$.

over the 2-yr follow-up was 0.96 step, with a median difference in dose of inhaled steroids of 400 μg per day (Figure 1).

Patients treated according to the AHR strategy had a lower incidence of mild exacerbations of asthma than those in the reference strategy group (0.23 and 0.43 exacerbations per year per patient, respectively). During 2 yr of follow-up, 60% (95% confidence interval [CI]: 41% to 74%) of patients in the reference strategy group experienced one or more such mild exacerbations whereas this was true for only 40% (95% CI; 24% to 56%) in the AHR strategy group (Figure 2). Applying a variant of the Cox regression that allows for multiple events, the exacerbation rate in the AHR strategy group was 1.8-fold (95% CI: 1.1 to 3.2) lower than in the reference strategy group.

The incidence of mild exacerbations of asthma was highest in those patients in the reference strategy group in whom AHR indicated a treatment step-up but who were not treated accordingly (0.52 exacerbations per year per patient) (Figure 3). In contrast, when the indication by AHR was put into prac-

TABLE 4

BASELINE LEVELS AND CHANGES FROM BASELINE TO 2 yr OF FOLLOW-UP IN SUBEPITHELIAL RETICULAR LAYER THICKNESS AND INFLAMMATORY CELL NUMBER IN BRONCHIAL BIOPSIES, AND THEIR RELATIONSHIP TO METHACHOLINE PC_{20}

Outcome Variable	Reference Strategy Mean (SD)	AHR Strategy Mean (SD)	Mean Difference	95% CI	p Value	Correlation Coefficient**
Reticular layer thickness						
Baseline, μm	8 (2.1)	8.6 (2.2)	−0.5	−1.8–0.8	0.41	−0.2
Change, μm	−0.4 (1.8)	−2.1 (2.6)‖	1.7	0.2–3.1	0.03	−0.09
$CD45^+$						
Baseline*	172 (1.87)	140 (1.62)	0.81‡	0.57–1.15	0.24	−0.29
Fold change¶	0.42 (2.33)‖	0.47 (2.72)‖	1.12‡	0.69–2.12	0.7	−0.34§
$EG2^+$						
Baseline*	8.8 (3.44)	8.3 (3.52)	0.94‡	0.45–2	0.88	−0.58‖
Fold change¶	1.1 (3.92)	0.81 (3.93)	0.74‡	0.3–1.85	0.51	−0.48‖
$AA1^+$						
Baseline*	15.5 (2.11)	23 (1.52)	1.48‡	1.03–2.14	0.04	−0.49§
Fold change¶	1.21 (2.47)	0.59 (2.48)§	0.49‡	0.26–0.91	0.03	−0.42§
$CD3^+$						
Baseline*	134 (1.88)	103 (2.0)	0.78‡	0.52–1.15	0.2	−0.3
Fold change¶	0.48 (2.45)‖	0.36 (4.18)‖	0.76‡	0.33–1.72	0.5	−0.42§
$CD4^+$						
Baseline*	58.4 (3.0)	31.4 (4.28)	0.54‡	0.25–1.17	0.12	−0.15
Fold change¶	0.24 (6.21)‖	0.45 (7.24)	1.85‡	0.51–6.78	0.34	−0.23
$CD8^+$						
Baseline*	61.5 (1.89)	50.4 (2.16)	0.82‡	0.53–1.26	0.36	−0.3
Fold change¶	0.23 (3.60)‖	0.34 (5.71)§	1.44‡	0.49–4.2	0.49	−0.33§
$CD45RO^+$						
Baseline*	177 (2.05)	162 (1.72)	0.91‡	0.61–1.37	0.66	−0.33
Fold change¶	0.5 (2.12)‖	0.52 (2.17)‖	1.05‡	0.61–1.79	0.86	−0.37§

§ $p < 0.05$.
‖ $p < 0.01$.
* Geometric mean number of cells/0.1 mm^2 (fold SD).
‡ Fold difference: AHR strategy/reference strategy.
¶ Fold change: outcome/baseline.
** Correlation coefficient between baseline (or change) in outcome variable and baseline (or change) in methacholine PC_{20}.

tice, the exacerbation rate was as low as in the patients not showing an indication by AHR (0.23 and 0.25 exacerbation per year per patient, respectively). The Cox regression (multiple events) showed that the exacerbation rate increased by 2.1-fold (95% CI: 1.0 to 4.3) in patients with a positive indication by AHR versus no indication by AHR.

Changes in mean prebronchodilator FEV_1 during the 2-yr follow-up are shown in Figure 4. As assessed by the area under the time-response curve, the improvement in FEV_1 was more pronounced with the AHR strategy than with the reference strategy (mean difference: 4.3 L · mo; 95% CI: 0.5 to 8.1) (Table 3). Furthermore, the variability in prebronchodilator FEV_1 in the AHR strategy group was smaller than in the reference strategy group (mean difference in individual SD = 46 ml; 95% CI: 2 to 93 ml). As expected, PC_{20} was significantly reduced, by 1.1 doubling dose (DD) (mg/ml), in the AHR strategy group (95% CI: 0.6 to 1.6 DD), whereas there was a nonsignificant reduction in the reference strategy group (0.46 DD; 95% CI: −0.4 to 1.3 DD).

In the bronchial biopsy specimens, the thicknesses of the subepithelial reticular layer at baseline for the reference and AHR strategy groups were 8.0 ± 1.6 μm (mean ± SD) and 8.6 (2.2) μm, respectively (Table 4). During the 2-yr follow-up, there was a significant decrease in thickness of the subepithelial reticular layer within the AHR strategy group, which was significantly greater than the change seen in the reference strategy group (mean difference: 1.7 μm; 95% CI: 0.2 to 3.1 μm) (Figure 5). Similar results were obtained when restricting the analysis to subjects who were already regularly using inhaled steroids at entry into the study (mean difference: 2.7 μm; 95% CI: 1.0 to 4.3 μm).

The cell counts in the lamina propria are listed in Table 4. At baseline there were no differences between the patients subjected to the two treatment strategies in inflammatory cell number, except for $AA1^+$ cells, being 1.5-fold more numerous in the AHR strategy than in the reference strategy group ($p = 0.04$). Both treatment strategies led to significant reductions in numbers of most of the subpopulations of leukocytes, particularly $CD3^+$, $CD4^+$, $CD8^+$, and $CD45RO^+$ cells (Table 4). However, these changes were not significantly different between the two treatment strategies, except for $AA1^+$ cells showing a greater reduction in the AHR strategy group (0.59-fold; 95% CI: 0.39 to 0.91). Interestingly, for most of the subpopulations of leukocytes, the change in cell number was related to the accompanying change in methacholine PC_{20} (Table 4). This was most marked for $EG2^+$ cells (Figure 6).

DISCUSSION

We believe that the present study has two messages. First, our results indicate that a strategy similar to that in the current international guidelines, in which the treatment steps for asthma are based solely on optimizing symptoms and lung function, does not lead to optimal control of asthma in each individual patient. Second, the degree of AHR in asthma provides relevant information on the exacerbation rate, which appears to be highest in patients with relatively severe hyperresponsiveness. Our findings suggest that a step-up in dose of inhaled steroids can be successfully tailored to the needs of the individual patient according to the degree of AHR, instead of applying an increased dose indiscriminately. In patients in whom AHR is treated through such a strategy, the rate of mild exacerbations of asthma decreases toward a level similar to that observed in patients without an indication for treating hyperresponsiveness. This can be achieved by a limited increase in dose of inhaled steroids. Such a treatment strategy also improves the level and variability of lung function, and, interestingly, leads to a significant reduction of thickening of the subepithelial reticular layer in the bronchial wall. This latter finding is indicative of a reversal of airway remodeling. Hence, monitoring AHR might be the first step toward improved long-term management of asthma.

To our knowledge, this is the first long-term randomized trial of the effect of adding a reduction of AHR to the current goals of asthma treatment. Similar randomized studies of treatment strategies are not available for the clinical markers of asthma that are included in the present guidelines. To date, the only studies that have been done have been those evaluating monitoring of the peak expiratory flow rate and symptoms in the (self)management of asthma (24, 25). These studies addressed the question of whether serial peak-flow measurements provide information additional to that provided by symptom diaries in detecting asthma exacerbations (24) or establishing the clinical severity of asthma (25). Taken together,

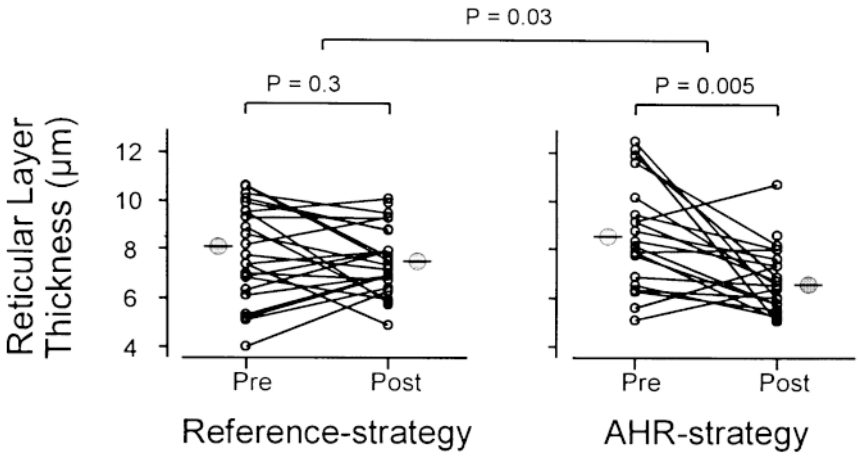

Figure 5. Individual changes in reticular layer thickness beneath the epithelium in bronchial biopsy specimens before and after 2 yr of treatment according to the reference and AHR strategies. *Bars* indicate mean values at the visits for both strategies. There was a significant decrease in reticular layer thickness within the AHR strategy group, which was significantly greater than in the reference strategy group.

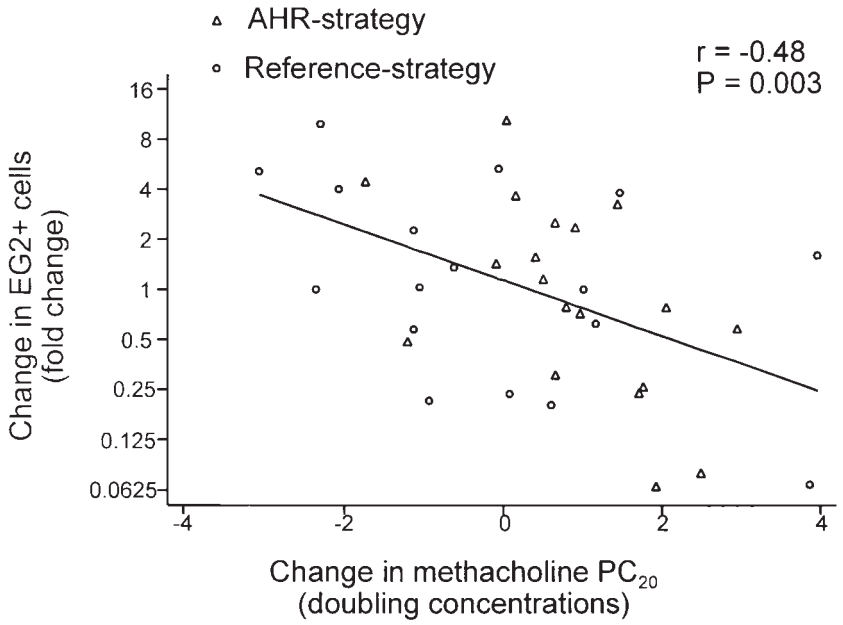

Figure 6. Relationship between changes in $EG2^+$ eosinophils and changes in methacholine PC_{20} during 2 yr of treatment according to the reference and AHR strategies. The greater the decrease in number of $EG2^+$ eosinophils, the greater the improvement in AHR to inhaled methacholine.

it seems that peak-flow monitoring is particularly valuable in patients with chronic severe asthma and/or impaired perception (26). We have extended this approach by including a new measurement in the definition of the control of asthma (27). Our data suggest that AHR indeed provides information complementary to that provided by the currently used clinical markers of the disease state in asthma.

The results of this study do not seem to have been influenced by differences in baseline characteristics, information bias, or inadequate treatment in the reference strategy group. First, there were no significant differences in baseline characteristics between the groups, except for the number of mast cells per 0.1 mm^2 in the bronchial mucosa. In particular, steroid-naive patients and patients already treated with inhaled steroids were balanced among the two arms of the study. Second, although complete double-blinding of the study could not be secured, since information on the patients' degree of AHR was presented to the chest physician in the AHR strategy group, the adjustment of the treatment level was determined by a strict algorithm. In addition, all outcome variables including the biopsies were obtained on coded material. Third, inadequate treatment of patients in the reference strategy group seems an unlikely explanation for the improved control of asthma in the AHR strategy group. The combined information from the diary cards and lung function measurements in the reference strategy group resulted in appropriate classification of asthma severity and corresponding treatment steps according to international guidelines (1). Additionally, we purposely limited the choice of antiasthma drugs to regular inhaled steroids and short-acting β_2-agonists taken as needed, in order to reduce the chance of drug bias between the two arms of the study.

How can we explain the improved control of asthma in the AHR strategy group? As could be expected, the AHR strategy resulted in a reduced degree of AHR to methacholine. This improvement was relatively limited (1.1 DD), which is not unexpected, because the majority of the patients had already been treated with inhaled steroids at baseline. It has been reported that a treatment-induced reduction in AHR in asthma was related to decreased cell numbers in the inflammatory infiltrate in the bronchial lamina propria (28, 29). This observation is in keeping with the view that AHR may serve as a surrogate marker of airways inflammation in asthma (10). Interestingly, most of the inflammatory cell numbers in our patients decreased in both arms of the study, although this was not true of all such cells (e.g., $EG2^+$ cells), again presumably because many patients were already taking inhaled steroids prior to the study.

It needs to be emphasized that AHR cannot be a marker of infiltration by a specific cell type, but rather is the phenotypic expression of multiple acute as well as chronic inflammatory events within the airways (30, 31). Our findings suggest that the latter property of AHR can be used in asthma management. The greater success in controlling asthma with the AHR strategy suggests that the reference strategy may not be sufficient in the long-term treatment of asthma. Under these circumstances, reversal of chronic inflammatory remodeling of the airways might be a more important goal of therapy. In this regard, we found that the thickness of the subepithelial collagen layer was reduced only with the AHR strategy, and toward a lower level than observed in the reference strategy group at baseline and the study endpoint. This indicates that the reticular layer thickness could potentially still be reduced in the reference strategy group. A reduction of the reticular layer thickness through the use of inhaled steroids has been the subject of controversy (28, 29, 32), and apparently requires doses that are guided by other markers than symptoms and lung function alone. This can be accomplished by temporary stepped increases in treatment, requiring doses of inhaled steroids that are considered to be safe ($\leq$ 800 μg/d) (33) in the great majority of subjects.

The clinical implications of the present findings seem to be fourfold. First, it appears that directing treatment at reducing symptoms and improving lung function does not lead to opti-

mal control of asthma in every patient. Hence, it appears that the current guidelines for asthma management may lead to suboptimal control of the disease in some patients. Second, it appears that such patients can be recognized from their increased levels of airway responsiveness. AHR can therefore be regarded as a marker of disease severity. Third, by including AHR in asthma management, the control of the disease can be improved. Over the long term, such a management strategy may not necessarily lead to increased steroid doses. It can even be postulated that introducing such a strategy at early stages of the disease may eventually reduce steroid requirements through improved clinical control (34). The observations in the present study also support the further development and validation of other noninvasive markers of inflammation, such as induced sputum (35) or exhaled air in the management of asthma. Testing for AHR in routine practice is relatively easy, and results can be obtained within 15 to 20 min. Therefore, monitoring of AHR seems to be the first step toward refining the long-term management of asthma.

References

1. National Institutes of Health, National Heart, Lung, and Blood Institute. 1995. Global initiative for asthma. Global strategy for asthma management and prevention. NHLBI/WHO Workshop Report. Publication No. 95-3695.
2. Djukanović, R., W. R. Roche, J. W. Wilson, C. R. W. Beasley, O. P. Twentyman, P. H. Howarth, and S. T. Holgate. 1990. Mucosal inflammation in asthma [State of the art]. *Am. Rev. Respir. Dis.* 142:434–457.
3. Roche, W. R., J. H. Williams, R. Beasley, and S. T. Holgate. 1989. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1:520–524.
4. Bousquet, J., P. Chanez, and J. Y. Lacoste. 1990. Eosinophilic inflammation in asthma. *N. Engl. J. Med.* 323:1033–1039.
5. Boulet, L. P., H. Turcotte, and A. Brochu. 1994. Persistence of airway obstruction and hyperresponsiveness in subjects with asthma remission. *Chest* 105:1024–1031.
6. Sont, J. K., J. H. J. M. van Krieken, C. E. Evertse, R. Hooijer, L. N. A. Willems, and P. J. Sterk. 1996. The relationship between the inflammatory infiltrate in bronchial biopsies and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 51:496–502.
7. Lange, P., J. Parner, J. Vesbo, P. Schnohr, and G. Jensen. 1998. A 15-years follow-up study of ventilatory function adults with asthma. *N. Engl. J. Med.* 339:1194–1200.
8. Woolcock, A. J. 1990. Assessment of bronchial responsiveness as a guide to prognosis and therapy in asthma. *Med. Clin. North Am.* 74:753–765.
9. Holgate, S. T. 1993. Asthma—past, present and future. *Eur. Respir. J.* 6: 1507–1520.
10. Szefler, S. J., and D. Y. M. Leung. 1996. Asthma management: past, present, and future. *In* S. J. Szefler and D. Y. M. Leung, editors. Severe Asthma: Pathogenesis and Clinical Management. Marcel Dekker, New York. 537–554.
11. Bradley, B. L., M. Azzawi, M. Jacobson, B. Assoufi, J. V. Collins, A. M. A. Irani, L. B. Schwartz, S. R. Durham, P. K. Jeffery, and A. B. Kay. 1991. Eosinophils, T-lymphocytes, mast cell, neutrophils, and macrophages in bronchial biopsy specimens for 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.* 88:661–674.
12. Cockcroft, D. W. 1991. Therapy for airway inflammation in asthma. *J. Allergy Clin. Immunol.* 87:914–919.
13. Woolcock, A. J. 1990. Use of corticosteroids in treatment of patients with asthma. *J. Allergy Clin. Immunol.* 84:975–978.
14. Pattemore, P. K., and S. T. Holgate. 1993. Bronchial hyperresponsiveness and its relationship to asthma in childhood. *Clin. Exp. Allergy* 23: 886–900.
15. Crimi, E., A. Spanevello, M. Neri, P. W. Ind, G. A. Rossi, and V. Brusasco. 1998. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am. J. Respir. Crit. Care Med.* 157:4–9.
16. Quanjer, Ph. H., G. J. Tammeling, J. E. Cotes, O. F. Pedersen, R. Peslin, and J. C. Yernault. 1993. Lung volumes and forced ventilatory flows. *Eur. Respir. J.* 6(Suppl. 16):S5–S40.
17. Hargreave, F. E., J. Dolovich, and M. T. Newhouse, editors. 1990. Rostrum: the assessment and treatment of asthma: a conference report. *J. Allergy Clin. Immunol.* 85:1098–1111.
18. Sterk, P. J., L. M. Fabbri, Ph. H. Quanjer, D. W. Cockcroft, P. M. O'Byrne, S. D. Anderson, E. F. Juniper, and J. L. Malo. 1993. Airway responsiveness: standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. *Eur. Respir. J.* 6:S53–S83.
19. Djukanović, R., J. W. Wilson, C. K. W. Lai, S. T. Holgate, and P. H. Howarth. 1991. The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am. Rev. Respir. Dis.* 143:772–777.
20. Workshop Summary and Guidelines. 1992. Investigative use of bronchoscopy, lavage and bronchial biopsies in asthma and other airways diseases. *Eur. Respir. J.* 5:115–121.
21. Sont, J. K., L. N. A. Willems, C. E. Evertse, P. J. Sterk, and J. H. J. M. van Krieken. 1997. Repeatability of measures of inflammatory cell number in bronchial biopsies in atopic asthma. *Eur. Respir. J.* 10:2602–2608.
22. Altman, D. G. 1991. Practical Statistics for Medical Research. Chapman and Hall, London. 430–433.
23. Lin, D. Y., and L. J. Wei. 1989. The robust inference for the Cox proportional hazards model. *J. Am. Stat. Assoc.* 84:1074–1078.
24. Malo, J. L., J. Larcheveque, C. Trudeau, C. Daquino, and A. Cartier. 1993. Should we monitor peak expiratory flow rates or record symptoms with a simple diary in the management of asthma? *J. Allergy Clin. Immunol.* 91:702–709.
25. Uwyyed, K., C. Springer, A. Avital, E. Bar-Yishay, and S. Godfrey. 1996. Home recording of PEF in young asthmatics: does it contribute to asthma management? *Eur. Respir. J.* 9:872–879.
26. Fishwick, D., and R. Beasley. 1996. Use of peak flow-based self-management plans by adult asthmatic patients. *Eur. Respir. J.* 9:861–865.
27. Cockcroft, D. W., and V. A. Swystun. 1996. Asthma control versus asthma severity. *J. Allergy Clin. Immunol.* 98:1016–1018.
28. Trigg, C. J., N. D. Manolitsas, J. H. Wang, M. A. Calderon, A. Mcaulay, S. E. Jordan, M. J. Herdman, N. Jhalli, J. M. Duddle, S. A. Hamilton, J. L. Devalia, and R. J. Davies. 1994. Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. *Am. J. Respir. Crit. Care Med.* 150:17–22.
29. Jeffery, P. K., R. W. Godfrey, E. Adelroth, F. Nelson, A. Rogers, and S. A. Johansson. 1992. 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.* 145:890–899.
30. Haley, K. J., and J. M. Drazen. 1998. Inflammation and airway function in asthma: what you see is not necessarily what you get. *Am. J. Respir. Crit. Care Med.* 157:1–3.
31. Olivieri, D., A. Chetta, M. Del Donno, G. Bertorelli, A. Casalini, A. Pesci, R. Testi, and A. Foresi. 1997. Effect of short-term treatment with low-dose inhaled fluticasone propionate on airway inflammation and remodeling in mild asthma: a placebo-controlled study. *Am. J. Respir. Crit. Care Med.* 155:1864–1871.
32. Chetta, A., A. Foresi, M. Del Donno, G. Bertorelli, A. Pesci, and D. Olivieri. 1997. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. *Chest* 111:852–857.
33. Kamada, A. K., S. J. Szefler, R. J. Martin, H. A. Boushey, V. M. Chinchilli, J. M. Drazen, J. E. Fish, E. Israel, S. C. Lazarus, and R. F. Lemanske. 1996. Issues in the use of inhaled corticosteroids. *Am. J. Respir. Crit. Care Med.* 153:1739–1748.
34. Haahtela, T., M. Järvinen, T. Kava, K. Kiviranta, S. Koskinen, K. Lehtonen, K. Nikander, T. Persson, O. Selroos, A. Sovijärvi, B. Stenius-Aarniala, T. Svahn, R. Tammivaara, and L. A. Laitinen. 1994. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N. Engl. J. Med.* 331:700–705.
35. in 't Veen, J. C. C. M., H. W. F. M. de Gouw, H. H. Smits, J. K. Sont, P. S. Hiemstra, P. J. Sterk, and E. H. Bel. 1996. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. *Eur. Respir. J.* 9:2441–2447.