JOUNI HEDMAN

# A Rapid Methacholine Challenge Test in Patients with Asthmatic Symptoms

*University of Tampere*
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ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the small auditorium of
Building K, Medical School of the University of Tampere,
Teiskontie 35, May 13th, 2000 at 12 o'clock.

*University of Tampere*
*Tampere 2000*

*To my family*

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# ABBREVIATIONS

| | |
|---|---|
| AHR | Airway hyperresponsiveness |
| AIA | Aspirin-induced asthma |
| ANOVA | Analysis of variance |
| ASM | Airway smooth muscle |
| ATS | American Thoracic Society |
| ATS-DLD | American Thoracic Society, National Heart, Lung and Blood Institute, Division of Lung Diseases |
| BAL | Bronchoalveolar lavage |
| BHR | Bronchial hyperresponsiveness |
| CI | Confidence interval |
| COPD | Chronic obstructive pulmonary disease |
| COX | Cyclo-oxygenase |
| CV | Coefficient of variation |
| DRS | Dose-response slope |
| ECCS | European Community for Coal and Steel |
| ECP | Eosinophil cationic protein |
| ERCHS | The European Community Respiratory Health Survey |
| EDN | Eosinophil-derived neurotoxin |
| EPX | Eosinophil protein X |
| $FEV_1$ | Forced expiratory volume in one second |
| FRC | Functional residual capacity |
| FVC | Forced vital capacity |
| IgE | Immunoglobulin E |
| IL-4 | Interleukin-4 |
| IL-5 | Interleukin-5 |
| ISAAC | International Study of Asthma and Allergies in Childhood |
| IUATLD | The International Union Against Tuberculosis and Lung Disease |
| L-ASA | Lysine acetylsalicylate |
| $LTE_4$ | Leukotriene $E_4$ |
| MPO | Myeloperoxidase |
| MRC | Medical Research Council |
| NSAID | Nonsteroidal anti-inflammatory drug |
| NO | Nitric oxide |
| OR | Odds ratio |
| $PC_{20}FEV_1$ | Provocative concentration causing a decrease of 20% in $FEV_1$ |
| $PD_{15}FEV_1$ | Provocative dose causing a decrease of 15% in $FEV_1$ |
| $PD_{20}FEV_1$ | Provocative dose causing a decrease of 20% in $FEV_1$ |
| PEF | Peak expiratory flow |
| r | Correlation coefficient |
| RR | Relative risk |
| SD | Standard deviation |
| SEM | Standard error of mean |
| TAS | The Tasmanian Asthma Survay |

# LIST OF ORIGINAL PUBLICATIONS

I Malmberg LP, Hedman J and Sovijärvi ARA (1993): Accuracy and repeatability of a pocket turbine spirometer: comparison with a rolling seal flow-volume spirometer. Clinical Physiology 13:89-98.

II Hedman J, Alanko K and Nieminen MM (1996): Repeatability of a rapid dosimetric method for methacholine challenge using a pocket turbine spirometer for $FEV_1$ measurements. Clinical Physiology 16:353-359.

III Hedman J, Poussa T and Nieminen MM (1998): A rapid dosimetric methacholine challenge in asthma diagnostics - A clinical study of 230 patients with dyspnea, wheezing or cough of unknown reason. Respir Med 92:32-39.

IV Hedman J, Kaprio J, Poussa T and Nieminen MM (1999): Prevalence of asthma, aspirin intolerance, nasal polyposis and COPD in a population based study. Int J Epidemiol 28:717-722.

V Hedman J, Moilanen E, Poussa T and Nieminen MM (1999): Serum ECP and MPO, but not urinary $LTE_4$, are associated with bronchial hyper-responsiveness. Respir Med 93:589-596.

 In addition some unpublished data have been included in the study.

# I INTRODUCTION

Bronchial hyperresponsiveness (heightened responsiveness of the bronchi to various physical, chemical and pharmacological stimuli, manifested as airway narrowing) was first demonstrated by Dautraband and Philipott, who introduced non-allergic bronchial inhalation challenges in 1941 (Banik and Holgate 1998). Curry (1946) subsequently demonstrated that histamine and acetyl-beta-methyl-choline (methacholine), when administered intravenously, could provoke airway narrowing in asthmatic but not in normal subjects

A methacholine or histamine challenge test is used to demonstrate nonspecific bronchial hyperresponsiveness found not only in asthmatics but also in patients with several other airway disorders as well as in healthy individuals (Cockroft et al. 1977, Mellis and Levison 1978, Laitinen et al. 1983, Nieminen 1992, Marcias et al. 1994, de Jong et al. 1997, Leone et al. 1997, Prieto 1998a). Both challenge tests constitute an elementary part of asthma diagnostics in clinical practice and in follow-up of the efficacy of treatment (Britton et al. 1986, Nieminen 1992, Sovijärvi et al. 1993, Sterk et al.1993, Sont 1999b).

Most asthmatic patients exhibit bronchial hyperresponsiveness in the methacholine test, but approximately 10 per cent have a negative test result when conventional methacholine doses up to 2600µg are used (Nieminen 1992). Larger cumulative methacholine doses might thus be appropriate when studying the less hyperresponsive end of the unimodal distribution of bronchial hyperresponsiveness to methacholine in asthmatic patients. Conventional challenge procedures are time–consuming, limiting their use especially in epidemiological surveys.

An intensive scrutiny of the mucosal inflammatory process has been under way, with one goal, namely to establish a simple marker for asthmatic inflammation, the asthma “sedimentation rate“ (Haahtela 1995). Multiple cellular and/or soluble markers of inflammation in peripheral blood, urine, hypertonic saline-induced sputum and exhaled air have been studied. These, however, reflect only certain aspects of inflammation (mostly eosinophilic inflammation), and the best means of monitoring airway inflammation may be a measure which is likely to be a result of the overall inflammation process (Sont 1999a). Sont and associates (1999b) have recently stressed the value of methacholine challenge as a physiological marker with heterogeneous pathophysiology in monitoring airway inflammation.

Previous large-scale epidemiological studies of the prevalence of asthma and COPD in the adult Finnish population were published at least 30 years ago (Huhti 1965, Alanko 1970). There is a marked overlap in symptoms between asthma and COPD, and the symptoms of patients with mild asthma are often erroneously taken to be caused by smoking. Hence population-based studies are needed, covering the whole range of asthmatic symptoms and diagnoses of obstructive pulmonary diseases, including detailed information on smoking habits.

Intrinsic asthma with the triad of nasal polyposis, aspirin intolerance, and asthma has been regarded as a different entity from extrinsic asthma, which is of allergic origin (Chafee and Settipane 1974, Settipane and Chafee 1977). The rate of decline in lung function is greater in patients with intrinsic than in those with extrinsic asthma, and the prognosis for intrinsic and extrinsic asthma is to some extent influenced by different factors, which also suggests that the pathogenetic mechanisms underlying the two forms may differ (Ulrik et al. 1992). There is no difference in baseline values for urinary $LTE_4$ levels between atopic asthmatics and non-asthmatic individuals (Kumlin et al. 1995), but higher urinary $LTE_4$ levels are reported in aspirin-sensitive as compared to aspirin-tolerant asthmatics and healthy controls (Smith et al. 1992, Sladek and Szczeklik 1993, Kumlin et al. 1995).

The aims of the present study was firstly to develop and assess a new, rapid, large-dose method using a Spira Elektro 2© dosimeter for methacholine delivery and a pocket turbine spirometer (Micro Spirometer©) for $FEV_1$ measurements, and also to evaluate the new method in the diagnostics of asthmatic symptoms. The further aim was to determine the prevalence of physician-diagnosed asthma, asthmatic symptoms and COPD in adults aged 18 - 65 years and to study the relationship of aspirin intolerance, nasal polyposis and allergic rhinitis to asthma as well as to study smoking habits in connection with respiratory symptoms. Thirdly, the validity of the Tuohilampi questionnaire items concerning cough with wheeze apart from cold, wheezing with shortness of breath (with breathing normal between attacks) and doctor-diagnosed asthma were examined as tests for bronchial hyperresponsiveness. Finally, a random population-based material was used to assess the value of serum MPO, ECP and urinary $LTE_4$ in indicating bronchial hyperresponsiviness as measured by methacholine challenge. Special interest was focused on a history of aspirin intolerance and on smoking as contributing factors.

# II REVIEW OF THE LITERATURE

## 1. Nonspecific bronchial responsiveness

Increased responsiveness or reactivity of the bronchi to various physical, chemical and pharmacological stimuli, manifested as airway narrowing, is known as bronchial or airway hyperresponsiveness/ hyperreactivity (Banik and Holgate 1998). Bronchial hyperresponsiveness to methacholine or histamine is closely associated with asthma, but is also found in several other inflammatory airway disorders, including allergic and non-allergic rhinitis, cystic fibrosis, chronic obstructive pulmonary disease, allergic alveolitis and sarcoidosis, as well as in healthy individuals (Cockroft et al. 1977, Nieminen 1992, Leone et al. 1997, Prieto 1998a, de Jong et al. 1997, Mellis and Levison 1978, Laitinen et al. 1983, Marcias et al. 1994). Patients with familial amyloidotic polyneuropathy and advanced autonomic neuropathy also evince bronchial hyperreactivity to methacholine and/or histamine, probably by reason of denervation supersensitivity resulting from amyloid deposition in the peripheral autonomic nerves of the airways (Kawano et al. 1997).

Constrictor stimuli can be divided into those which act predominantly through a direct effect on airway smooth muscle, for example histamine (which acts on $H_1$ receptors) and methacholine (which acts on the $M_3$-muscarinic receptor) and those acting indirectly by stimulating neural pathways or release of inflammatory mediators, for example isocapnic dry air hyperventilation, exercise, inhalation of adenosine monophosphate (AMP) or sodium chloride (Banik and Holgate 1998, Lotvall et al. 1998, Anderson et al. 1997). Within the asthmatic population AMP challenge may not provide different information from that obtained by histamine challenge; e.g. both reflect the same pathophysiological processes in the airways (Egbadge et al. 1997). It is, however, conceivable that different challenge methods might be used to understand divergent aspects of the underlying mechanisms of the change in AHR induced by allergen exposure (Lotvall et al. 1998). Inhaled frusemide exerts a protective effect against bronchoconstriction induced by several indirect stimuli (including neurokinin A) possibly due to interference with airway nerves (Crimi et al. 1997).

### *1.1. Definition of terms*

Airway responsiveness to bronchoconstrictor stimulus is expressed as the provocative dose ($PD_{20}$) or concentration ($PC_{20}$) of the stimulus required to achieve a given level of bronchoconstriction (typically a 20% fall in $FEV_1$). A decrease in $PC_{20}$ or $PD_{20}$ may be due to a steeper dose-response curve (hyperreactivity) or to a shift in the curve to the left (hypersensitivity), or both. When an individual has diminished $PC_{20}$ or $PD_{20}$ it is usually not known whether

this is due to hyperreactivity or hypersensitivity or both, and the term which covers both, airway "hyperresponsiveness" is preferred (Lotvall et al. 1998).

Such airway reactivity can also be expressed as a dose-response slope (DRS) obtained by dividing the achieved percentage change in $FEV_1$ by the cumulative dose (µmol) of methacholine used (O`Connor et al. 1987). DRS can be calculated for all patients in all conditions, and no data will be lost as a result of limited change in pulmonary function (Seppälä et al. 1998). Results from a random population study have shown that DRS values, which could be obtained for most subjects, contributed additional information to $PD_{20}FEV_1$ values and discriminated more accurately between groups classified according to respiratory history (Peat et al. 1992). In subjects in whom a $PD_{20}FEV_1$ could not be measured, the DRS bore a significant relation to asthma symptoms, smoking history and $FEV_1$/FVC.

## *1.2. Epidemiology*

A basic problem in studies dealing with asthmatic symptoms is the absence of any gold standard for the diagnosis of asthma (Toelle et al. 1992). Even when lung function studies are included, the variable nature of the disease makes it difficult to verify the diagnosis in epidemiological studies as well as in clinical practice. The importance of a careful history, spirometry, peak flow monitoring and methacholine challenge is stressed in the diagnostics (Burr 1992, Taylor 1997). Although the presence of diagnosed asthma and asthma-like symptoms is best predicted by the methacholine test, measurement of PEF variability might identify a different range of airway pathology (Siersted et al. 1996).

Attempts to compare bronchial responsiveness between populations have been hampered by between-study differences in the pharmacological agent of provocation, the method of administration and the summary statistic employed. In The European Community Respiratory Health Survey (ECRHS) methacholine challenge delivered by Me.far dosimeter according to a standardised protocol was used (Chinn et al. 1997b). Responsiveness was low in Iceland (7.2%) and Switzerland (9.8), and in most centres in Sweden (7.7-11.8%), Italy (9.3-11.6%) and Spain (3.4-21.3%), and high in New Zealand (22.7-27.8%), Australia (22.0%), the USA (18.3%), Britain (15.5-27.6%), France (12.0-23.2%), Denmark (23.5%) and Germany (12.0-17.5%). In Sweden a trend towards a higher prevalence of BHR was found in the most northerly of the study areas, but regional differences were not statistically significant (Norrman et al. 1998).

Prevalences of hyperresponsivenes for methacholine in recent adult population studies are summarised in Table 1.

***Table 1.*** *Prevalence of hyperresponsiveness to methacholine in adult population studies*

| Author | Age | Cut-off value | Prevalence (%) | N |
|---|---|---|---|---|
| Trigg et al. 1990a | 18-75 | 2mg/ml | 23 | 318 |
| Neukirch et al. 1992 | 22-58 | 1.2 mg | 16.2 | 117 |
| Higgins et al. 1993 | 18-65 | 24.5 µmol | 22.3 | 202 |
| Boezen et al. 1996 | 20-70 | 2 mg | 24.1 | 399 |
| Nowak et al. 1996 | 20-44 | 2 mg | 25 (Hamburg) | 934 |
| | | | 19 (Erfurt) | 593 |
| Devereux G et al. 1996 | 20-44 | 1.0 mg | 17.5 (West Cumbria) | 285 |
| | | | 15.6 (Newcastle) | 302 |
| | | 6.4 mg | 27.7 (West Cumbria) | 285 |
| | | | 28.2 (Newcastle) | 302 |
| Chinn et al. 1997b | 20-44 | 1.0 mg | 13.0 (median of 16 countries) | 13161 |
| Norrman et al. 1998 | 20-44 | 1.0 mg | 12.7 | 1448 |

### *1.2.1. Questionnaire studies*

In questionnaire studies, when validated in relation to bronchial challenge tests, questions on "physician-diagnosed asthma" have been shown to have very high specificity (up to 99 percent), which is especially important when comparisons are made of prevalences between different populations (Torèn et al. 1993). A wide geographical variation in the prevalence of physician-diagnosed asthma was found in the ECRHS, the highest figures being in New Zealand and Australia, 11-13% and lowest in Erfurt, Germany, 1.2% and Spain, 1.5-3.0% (Janson et al. 1997b). Jenkins and colleagues (1996) investigated the validity of The Tasmanian Asthma Survey (TAS) and the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaires by comparing response to questionnaire with a physician`s assessment of asthma status in the preceding 12 months. In both adults and children, questionnaires showed high agreement with physician diagnosis with respect to asthma symptoms in the preceding 12 months. Compared to the physician diagnosis, the sensitivity of bronchial hyperresponsiveness (BHR) for asthma was low for adults 0.39 (0.21-0. 61) and children 0.54 (0.48-0.67), as were the positive predictive values: 0.55 (0.31-0.79) for adults and 0.64 (0.449-0.77) for children.

It is also recommended in epidemiological studies to ask subjects about the cardinal symptoms of asthma rather than the diagnosis (Burr 1992). Toelle and associates (1992) have proposed that for epidemiological purposes current asthma should be defined as appropriate symptoms in the previous 12 months together with evidence of increased responsiveness to histamine. In the ECRHS the median for wheeze and breathlessness was 9.8%, prevalences varying from 3.0 % in Bombay to 16.3% in Caerphilly, and the median for wheeze with no cold 12.7%, prevalences varying from 2.0% in Bombay to 21.7% in Dublin. The

median prevalence of nasal allergy was 20.9%, prevalences varying from 9.5% in Algiers to 40.9% in Melbourne (Burney et al. 1996).

In the International Union Against Tuberculosis and Lung Disease (IUATLD) Bronchial Symptoms Questionnaire the most sensitive item for predicting hyperresponsiveness was the question on wheeze, sensitivity 0.59-0.95, and the most specific questions were those on waking at night with shortness of breath, specificity 0.74-0.83, and morning tightness, specificity 0.57-0.93 (Burney et al. 1989b). The format did not, however, distinguish the reactivity associated with smoking in older subjects from that associated with atopy in younger subjects (Burney et al 1989a). IUATLD questionnaire items predicting asthma syndrome were those referring to wheeze at rest or following exercise, asthma attack, chest tightness and shortness of breath at rest. Questions on coughing identified a different group of subjects who did not have asthma (Bai et al. 1998).

A standardized questionnaire on respiratory symptoms provided no adequate information to discriminate between those with and without BHR (histamine challenge) in a population sample of 551 subjects aged 10-23 years (Kolnaar et al. 1995). In that study BHR was present in 42% of subjects, of whom up to 70% were asymptomatic. Moreover, respiratory symptoms did not identify adults (aged 45-86 years) with airflow obstruction or bronchial hyperresponsiveness measured by methacholine challenge in the study by Renwick and Conolly (1999). Of subjects with bronchial hyperresponsiveness, 26.4% were asymptomatic. In men and women aged 65 years or more symptoms of the "bronchial irritability syndrome" were more strongly associated with airways lability (measured by methacholine challenge or salbutamol) than other symptoms, but their predictive value for airways lability was low (32%) in a study by Dow and colleagues (1992).

### *1.3. Risk factors*

In young adults atopy is the most important risk factor underlying bronchial responsiveness, and in the UK sensitisation to house dust mite and Cladosporium has been shown to be the most prominent individual risk factor (Chinn et al 1998b). In hyperreactive young adults in Australia parental asthma, keeping pets during childhood, allergy to house dust mite, allergic rhinitis, and having at some time smoked were associated with an increased risk of wheeze (Dharmage et al. 1998). In Sweden cats and dogs were the sensitising allergen sources most closely associated with asthma and BHR. The relationships with sensitisation to grass and mites were less pronounced (Plaschke et al. 1999).

In a random sample of the adult population in Western Australia (Woolcock et al. 1987), the total prevalence of bronchial hyperresponsiveness was 11.4% (measured by the response to histamine or in subjects with poor lung function by response to a bronchodilator). In that study, the distribution of bronchial hyperresponsiveness was continuous. There was a significant association between BHR and respiratory symptoms, atopy, smoking and abnormal lung function. In a study of Renwick and Conolly (1997) involving adults aged 45 or

older, airway calibre and level of bronchial responsiveness were associated with a defined standardised IgE score.

In a random sample of subjects aged 15-72 years more abundant eosinophils, skin test positivity and living in a rural area (Vlagtwedde, The Netherlands) were associated with increased responsiveness to histamine, independently of the level of $FEV_1$ and the presence of respiratory symptoms (Rijcken et al. 1993). Older age was associated with increased responsiveness, and this was even more so in subjects with symptoms. Cigarette smokers were more responsive than nonsmokers, but this association was not significant if the level of $FEV_1$ was taken into account. Bronchial hyperresponsiveness to histamine appeared to be age-dependent in an earlier population study by Rijcken and colleagues (1987) in The Netherlands, the proportion of responders increasing from 13% in those 14 to 24 yr of age to 40% in those 55 to 64 yr of age. Regardless of smoking history, responders were more likely to be symptomatic than were nonresponders (Odds ratios ranged from 1.7 for chronic cough to 4.4 for asthmatic attacks). In the study by de Marco and group (ECRHS, 1998), the main risk factors for BHR were respiratory symptoms and atopy, while younger age and larger airway calibre exerted a protective action. Their results also suggested that in epidemiological surveys 2 mg methacholine would suffice to fully evaluate the effect of risk factors on BHR.

Smokers evince greater bronchial responsiveness to methacholine, this, however, possibly only among non-atopic individuals (Sunyer et al. 1997). Smoking seems not to increase responsiveness in atopic subjects, which can, however, be caused by self-selection bias (Weiss and Sparrow 1989). Outdoor air pollutants may aggravate respiratory symptoms as well as increase responsiveness of the airways to methacholine and allergens (Sandström 1995).

## *1.4. Genetics*

The mechanism of genetic susceptibility to bronchial hyperresponsiveness is unknown. Large-scale mapping of the human genome is under way with a view to identify candidate genes for asthma, bronchial hyperresponsiveness and atopy. There are multiple regions of the genome which are likely to contain susceptibility genes for asthma and associated phenotypes which include BHR and atopic parameters (Howard et al. 1999). It is likely that susceptibility to develop asthma is attributable to multiple genes interacting with each other and with environmental factors to determine the expression of the asthmatic and atopic phenotype (Bleecker et al. 1997). A study by Laitinen and associates (1998) indicates that the presence of asthma in successive generations is more likely caused by shared genes than shared environmental risk factors. Substantial heterogeneity among families may, however, exist. A significant familial predisposition to BHR among patients with allergic rhinitis has also been observed (Koh et al. 1998).

A study by Postma and colleagues (1995) demonstrated that a trait entailing an elevated level of serum total IgE is coinherited with a trait for bronchial hyperresponsiveness, and that a gene governing bronchial hyperresponsiveness is

located near a major locus which regulates serum IgE levels on chromosome 5q. On the other hand, IL-4 promoter C-590T (gene located in chromosome 5) polymorphism may be associated with the development of asthma in Japanese children, but not through modulating total serum IgE levels (Noguchi et al. 1998).

Doull and associates (1996) have shown that on chromosome 11q, allele 168 at the D11S527 locus is significantly associated with BHR but not with log IgE. At the D11S534 locus, allele 235 was significantly associated with log IgE but not with BHR. These studies provide support for the view that both chromosomes 5 and 11 may contain genes relevant to asthma and atopy, a possible candidate being the interleukin-4 (IL-4) gene cluster (Doull et al. 1996). On the other hand, polymorphisms in the $\beta_2$-receptor gene on chromosome 5q32 have proved to be associated with differences in airway hyperresponsiveness (Ramsay et al. 1999). In a population study by D`amato and colleagues (1998), an association of persistent BHR and $\beta_2$-receptor gene haplotype with a Gly at position 16 and a Gln at position 27 was observed.

Amelung and coworkers (1998) studied markers in the area of the high-affinity IgE receptor (FcepsilonRI-beta) on chromosome 11q (D11S1314, FcepsilonRI-beta and D11S987) and were unable to confirm the presence of significant mutations in FcepsilonRI-beta gene in a Dutch population, nor could they confirm that the FcepsilonRI-beta gene is crucial to the pathogenesis of allergic inflammation in asthma.

## *1.5. Mechanisms*

Airway calibre is the result of a balance between the force generated by the airway smooth muscle (ASM) and a number of opposing factors, which are mainly represented by autonomic mechanisms tending to limit ASM tone and mechanical forces opposing ASM shortening. Figure 1 summarises the hypothetical mechanisms and pathways of airway hyperresponsivenes, as suggested by Brusasco and associates (1998).

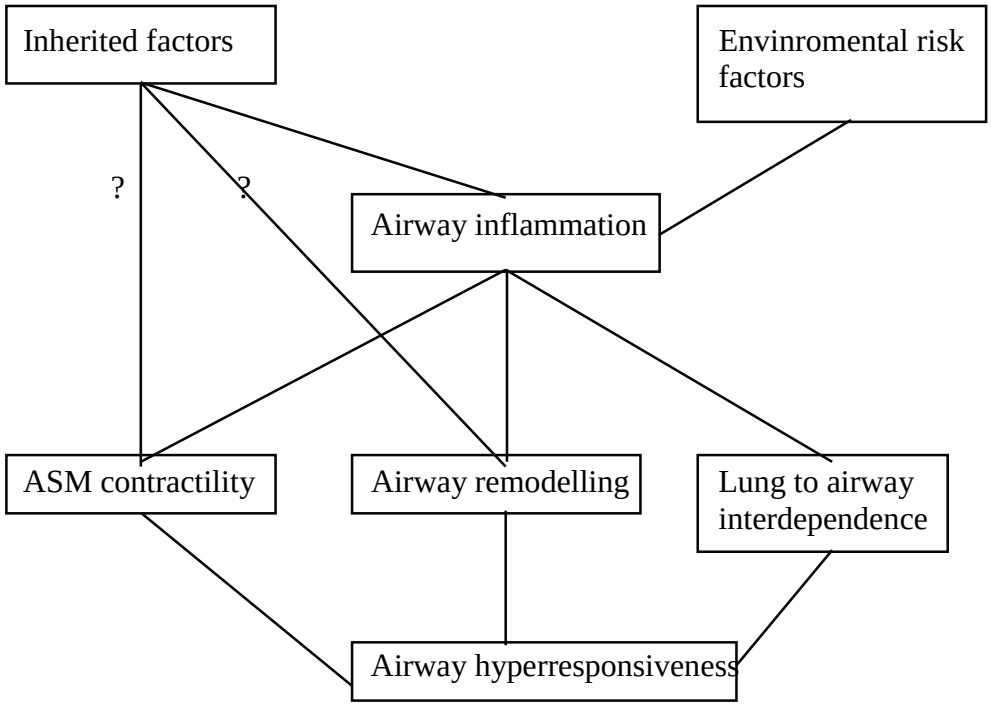


***Figure 1.*** Hypothetical mechanisms and pathways of airway hyper-responsiveness. Modified from Brusasco et al. (1998).

### *1.5.1. Inflammatory processes*

Asthma is characterised by chronic inflammatory changes in the airway mucosa even in the mildest form of the disease (Laitinen et al. 1985, Laitinen et al. 1993). The infiltration of inflammatory cells (eosinophils, macrophages and lymphocytes) in the lamina propria of the airways of asthmatic patients has been shown to be inversely related to $PC_{20}$ for methacholine (Sont et al. 1996), while in atopic subjects with mild to moderate asthma no correlation could be found between the degree of airway responsiveness and the numbers of inflammatory cells in sputum or bronchoalveolar lavage or bronchial biopsy (Crimi et al 1998). Inflammatory cells can modify airway responses at least by releasing mediators such as histamine, leukotrienes, platelet-activating factor and various proteases, or by releasing of cytokines and chemokines (Haley and Drazen 1998).

The pathogenesis of hyperreactivity is unclear. It may be related to increased production of cytokines such as IL-4 (Shi et al. 1998) or IL-5 (Ackerman et al. 1994, Tang et al. 1996), or to epithelial injury by products of eosinophils (Jeffery et al. 1989) with the consequent loss of the epithelial barrier (Davies and Devalia 1992). IL-4 increases airway responsiveness by recruiting eosinophils into the airway in allergic asthma (Shi et al. 1998). Cytokines directly reduce ASM responsiveness to ß adrenergic agents, stimulate cytokine secretion, inhibit or promote ASM proliferation and prime ASM to become hyperresponsive to bronchoconstriction (Amrani and Panettieri 1998). Serum interferon-gamma levels correlate with $PC_{20}$ and with circadian PEF variation in atopic asthmatics (ten Hacken et al. 1998). Cytokines may also act directly or indirectly on ASM

cells and alter myocyte function by modulating contractile agonist-induced calcium signalling in human ASM cells (Amrani and Panettieri 1998). There is a strong positive correlation between bronchial reactivity and the level of intracellular magnesium; magnesium intervenes in calcium transport mechanism and intracellular phosphorylation reactions (Dominguez et al. 1998). Respiratory virus infections increase airway hyperresponsiveness in conjunction with augmented airway inflammation (Grunberg et al. 1997).

### *1.5.2. Airway wall thickening*

In addition to inflammation, an important pathophysiological feature of asthma is a remodelling of the airways involving an increase in ASM mass, disruption of the airway epithelium and changes in the airway tissue extracellular matrix. The thickening of the subepithelial layer in asthma is due to an increase in fibroblasts, and the thickness of the subepithelial collagen appears to be linked to an increase in bronchial responsiveness (Hoshino et al. 1998a). In addition, exudation of plasma can cause oedema, and thus thickening of the airway wall (Persson 1986). By a geometric mechanism (ASM shortening) thickening of the airway wall can enhance the airway luminal resistance (Hogg et al. 1987). Airway hyperresponsiveness may be present even in the absence of demonstrable inflammatory cells in the airway lumen or mucosa (Foresi et al. 1997).

In normal subjects the response to methacholine is greatly enhanced by breathing just 500 ml below functional residual capacity (FRC), suggesting that an intrinsic impairment of the ability of inspiration to stretch airway smooth muscle is a major feature of asthma (Skloot et al. 1995). By comparing the responses to methacholine of asthmatic and control subjects, however, Burns and Gibson (1998) have shown that hyperresponsiveness of asthmatic airways is not attributable simply to an inability of deep inspiration to stretch airway smooth muscle.

Using high-frequency input impedance measurements Frey and colleagues (1998) could show that the flow limitation during methacholine challenge in infants is determined by a decrease in airway wall compliance. It is also possible to demonstrate reversible airway obstruction caused by methacholine challenge in the cross-sectional area of small airways by means of helical thin-section computed tomography (Goldin et al. 1998).

### *1.5.3. Maximal dose-response*

The dose-response curve reaches a maximum (plateau) in individuals with normal or mildly increased airway sensitivity. The presence of plateau response is assumed to indicate a limit to the degree to which airways can narrow, while moderate to severe asthma is characterised by the absence of such a limitation to narrowing (Woolcock et al. 1984, Sterk et al. 1985). The plateau response is a subject characteristic which is independent of the method of inhalation challenge testing, but repeatability of the plateau is low (Lougheed et al. 1993). It is, however, rarely possible to measure the maximal response in clinical studies and never in epidemiological studies, where a high response rate is required (Chinn 1998a).

In normal subjects the maximal activation of ASM is balanced by an equal afterload at the maximal dose-response plateau, e.g. progressive hyperinflation and/or parenchymal stiffening increases the parenchymal load and attenuates further airway narrowing (Moore et al. 1998). Airway hyperresponsiveness could thus result from a failure of afterload to attenuate muscle shortening after maximal activation (Moore et al. 1998).

# 2. Methacholine challenge

## *2.1. Different methods*

Recommendations for the standardisation of methacholine and histamine bronchial challenges were issued in 1983 (Eiser et al.) and updated in 1993 (Sterk et al.). Neither document recommended a single protocol, and it also seems unlikely that researches or clinicians could agree to standardise the measurement of BHR. In fact, the use of $FEV_1$ is virtually the only point on which testing in adults is standardised (Chinn 1998a). The widely used variety of techniques for the measurement of nonspecific bronchial hyperresponsiveness are summarised in Table 2.

***Table 2.*** *Summary of widely used techniques for the measurement of nonspecific bronchial hyperresponsiveness*

| Method | Drug | Maximal dose or concentration | Nebuliser |
|---|---|---|---|
| a) Tidal breathing methods | | | |
| Cockcroft et al. 1977 | histamine | 8.0 mg/ml | Wright`s nebuliser |
| b) Dosimeter methods | | | |
| Chai et al.1975 | histamine | 32 mg/ml | De Vilbiss No 42 nebuliser and |
| | methacholine | 32 mg/ml | Rosenthal-French dosimeter |
| Nieminen et al. 1988 | methacholine | 2.3 mg | Spira Elektro 2 |
| Sovijärvi et al. 1993 | histamine | 1.6 mg | Spira Elektro 2 |
| Chinn et al. 1997b | methacholine | 1.0 mg (1) | Me.far |
| (ERCHS) | methacholine | 2.0 mg (2) | Me.far |
| c) Yan method (a hand-operated method, delivering aerosols during inspiration only) | | | |
| Yan et al. 1983 | histamine | 3.9 μmol | De Vilbiss No 40 nebuliser |
| Higgins et al. 1988 | methacholine | 12 μmol | De Vilbiss No 40 |

1 mol methacholine chloride = 195.4 g (Sterk et al. 1993)

ERCHS = the European Community Respiratory Health Survey:
(1) Method 1, (2) Method 2

Histamine and methacholine inhalations seem to provoke approximately equal extents of bronchial obstruction in asthmatic and bronchitis patients (Laitinen 1974), and the two agents may be used with equal effectiveness in bronchial challenges (Salome et al. 1980). Juniper and colleagues (1978) suggested that methacholine has a small cumulative effect but that the effect of histamine is non-cumulative. In epidemiological studies methacholine is a more sensitive test for nonspecific bronchial reactivity than histamine, with fewer undesirable effects. Methacholine results were also slightly more repeatable in a study by Higgins and group (1988).

Factors affecting airway responsiveness are summarised in Table 3 (adapted from James and Ryan 1997).

***Table 3.*** *Factors affecting airway responsiveness*

| Technical | Non-technical |
|---|---|
| Preparation of solutions | Sex |
| Nebuliser output | Age |
| Droplet size | Body size |
| Breathing pattern | Allergen exposure |
| Measurement of response | Baseline lung function |
| | Smoking |
| | Drug treatment |
| | Diurnal variation |
| | Viral infection |

Adapted from James and Ryan (1997)

## *2.2. Reliability (Reproducibility)*

### *2.2.1. Within-testing protocols*

Responses to histamine and methacholine have been shown to be highly reproducible (coefficient of determination =0.994 and 0.990 respectively) when a tidal breathing method was used (Juniper et al. 1978). The repeatability of a dosimetric methacholine challenge test (dosimeter Me.far MB3, cumulative dose 3.2 mg) was studied by Balzano and associates (1989), and the 95 % confidence intervals (as based on a single determination) corresponded +/- 1.66 fold-difference in $PD_{20}$ from one visit to the other (three separate occasions in one week). In a study by Ryan and group (1981), using a De Vilbiss 646 nebuliser attached to a Rosenthal-French Dosimeter Model B-2A, the 95% confidence interval of $PC_{20}$, based on a single determination, was approximately the observed value +/- a two-fold concentration difference. In a study by Inman and colleagues (1998) methacholine airway responsiveness was measured on two occasions (separated by 35+/- 17 days), and the reproducibility of the $PC_{20}$ was high (intraclass correlation coefficient=0.94).

In mild asthmatics there were no significant differences in methacholine challenge results obtained on tests at 24 h intervals over a period of 5 days and no evidence for the development of tolerance to methacholine at one-day intervals. The 95% confidence interval for repeatability of the results was +/- 1.05 doubling doses of methacholine (Trigg et al. 1990b).

### *2.2.2. Between-testing protocols*

In the study by Juniper and colleagues (1978) already mentioned, responsiveness to histamine correlated closely with responsiveness to methacholine. Bennett and Davies (1987) compared in asthmatics bronchial challenge with histamine and methacholine for the tidal breathing method using the DeVilbiss 646 nebuliser and the dosimeter method using in addition the Rosenthal-French dosimeter. There was a significant difference between the $PC_{20}$ $FEV_1$ but not the $PD_{20}$ $FEV_1$ when either substance was administered by the different techniques. In a study of Beach and colleagues (1993) the coefficient of repeatability (and hence precision) for the measurement of airway responsiveness was significantly better by the dosimeter method (3.0) than by conventional Wright nebuliser tidal breathing method (10.9), but the technique for quantifying $FEV_1$ contributed more to this than that for delivering methacholine. Britton and colleagues (1986) detected no significant difference in repeatability between three different histamine challenge methods (Yan, Cockcroft and Mortagny).

It is practical as well as desirable to compare the precision of different techniques for the measurement of airway responsiveness and to derive conversion factors with an eye to equaling results (Beach et al. 1993).

### *2.2.3. The effect of subject experience*

The repeatability of methacholine challenge is likely to improve with practice, and laboratory-based studies on experienced subjects may overestimate the repeatability of a test in inexperienced subjects. A study by Knox and associates (1991) showed that differences in the repeatability of methacholine challenge between the Yan and dosimeter methods were small. Values obtained in experienced subjects, however, showed a better repeatability than those obtained in inexperienced subjects.

### *2.2.4. Nebuliser*

Calculation of dose requires a measure of nebuliser output. The active aerosol component in nebulisers is less than 100% of output by weight, and may vary between nebulisers in different batches from the same manufacturer (Chinn et al.1997a). At best the calculated dose is a good approximation to the dose delivered to the upper airway, but does not necessarily represent that reaching the lungs (Chinn 1998a). The deposition of particles in the lungs is determined by the mode of inhalation, particle or droplet size, and the degree of airway obstruction. When nebulisers are used the deposition depends primarily on the choice of nebulisers with relatively small droplet size and on the volume fill and compressed gas flow rate (Newman 1985).

### *2.2.5. Tolerance or tachyphylaxis*

Diurnal variation is not likely to exert an important confounding effect on methacholine tests in asthmatics carried out between 08:00 hours and 20:00 hours, but confounding could result from refractoriness if tests are repeated at intervals up to 24 hours (Beach et al. 1995). Beckett and colleagues (1992) , however, found no marked tolerance to methacholine in mild asthmatic patients with multiple repeated challenges over 6 h compared with normal subjects who demonstrated significant tolerance. The relatively low cumulative dose of methacholine required in asthmatic patients to produce obstruction may be insufficient to produce tolerance.

### *2.2.6. Other sources of bias*

Log $PC_{20}FEV_1$ is directly correlated to baseline pulmonary function (Fujimura et al. 1993a). The data of O`Connor and associates (1994) provided lower limits of normal $PD_{20}FEV_1$ which are specific for a subject's prechallenge $FEV_1$; however, these $FEV_1$-specific lower limits of normal $PD_{20}FEV_1$ provided no greater sensitivity and specificity for detecting asthma and wheezing than did a single lower limit of normal $PD_{20}FEV_1$ for all subjects.

Analysis of percentage $PD_{20}$ below an arbitrary cut-off point by logistic regression might be misleading, given the unimodal distribution of BHR in the population; it lacks power, and is unhelpful for those wishing to combine results using meta-analysis (Chinn 1998a). Instead the use of least square slope, which uses all information, has been recommended for epidemiological studies (Abramson et al. 1990).

# 3. Airway responsiveness in asthma

## *3.1. Airway inflammation*

Inflammation has been shown to be an important aspect of asthma pathogenesis, and it is present even at a clinically early stage of the disease (Laitinen et al. 1993). Airway inflammation and bronchial hyperresponsiveness do not, however, always correlate (Crimi et al.1998). Fabbri and colleagues (1988) suggested that there are at least two components in airway hyperresponsiveness: a transient one, which is caused by airway inflammation, and a long-lasting one, which is unrelated to acute inflammatory stimuli. Crimi and group (1998) suggested that factors other than inflammation (e.g. airway wall remodeling or autonomic dysfunction) may be responsible for most of the individual variability of airway responsiveness in asthma.

The infiltration of inflammatory cells in the lamina propria of the airways in asthmatic patients was inversely related to $PC_{20}$ for methacholine in a study by Sont and coworkers (1996). Chetta and associates (1996) also showed that

eosinophilic inflammation of the bronchial epithelium correlated with methacholine responsiveness in asthma. They suggest, moreover, that remodeling of the airways as e.g. thickening of the subepithelial layer correlates with indices of asthma severity and could contribute to the degree of methacholine but not to ultrasonically nebulised distilled water responsiveness. Boulet and group (1997) found in asthmatics a significant correlation $PC_{20}$ and subepithelial fibrosis intensity. In that study, the degree of subepithelial fibrosis did not correlate with the baseline $FEV_1$.

### *3.2. Markers of eosinophilic inflammation*

Multiple cellular and/or soluble markers of inflammation have been studied in peripheral blood, urine, hypertonic saline-induced sputum and exhaled air. Intensive scrutiny of the mucosal inflammatory process has consistently sought to establish a simple marker for asthmatic inflammation, the asthma "sedimentation rate" (Haahtela 1995). Such markers, however, reflect only certain aspects of inflammation (mostly eosinophilic inflammation), and the best means of monitoring airway inflammation may be a measure which can be assumed to be a result of the overall inflammation process (Sont 1999a).

Horn and colleagues (1975) demonstrated an inverse correlation between the level of pulmonary function and the number of blood eosinophils in adults with intrinsic asthma. Studies in both childhood and early adulthood asthmatics showed a relationship between blood eosinophilic cell count and severity of asthmatic symptoms, level of pulmonary function and histamine responsiveness (Ulrik 1995). Increased airway responsiveness to methacholine is associated with eosinophil counts in subjects with chronic respiratory symptoms, and asymptomatic subjects with increased airway responsiveness also show increases in eosinophil counts (Annema et al. 1995).

Myeloperoxidase (MPO), as a parameter of neutrophil activity, and eosinophil cationic protein (ECP), as a parameter of eosinophil activity, are both elevated in induced sputum in patients with asthma and COPD (Keatings and Barnes 1997a, Yamamoto et al. 1997). Serum ECP is also thought to be a useful marker of eosinophilic inflammation in bronchial asthma (Niimi et al. 1998). A higher level of serum ECP in acute asthma exacerbation is associated not only with more severe exacerbation but also with a lower degree of bronchodilator response (Lee et al. 1997). Serum ECP and MPO are elevated in children with persistent asthma symptoms (Kristijansson et al. 1994, Carlsen et al. 1997). Further, serum ECP, but not serum MPO, is influenced by atopy and eczema states (Kristijansson et al. 1994, Carlsen et al. 1997). Serum ECP has also been shown to correlate with the percentage of eosinophils in bronchoalveolar fluid and in bronchial biopsy specimens, and reflects the intensity of eosinophil airway inflammation as well as disease activity (Niimi et al. 1998).

Jatakanon and associates (1998) showed a significant correlation between exhaled NO, and $PC_{20}$ for methacholine on one hand, sputum eosinophils (%) on the other, and also between sputum eosinophils (%) and $PC_{20}$. Treatment of asthmatic subjects with inhaled fluticasone propionate (500 μg twice daily) for

four weeks led to improvements in airway hyperresponsiveness to histamine, eosinophil counts in induced sputum, and exhaled nitric oxide levels (van Rensen et al. 1999).

Sputum cysteinyl-leukotriene concentrations were shown to be significantly higher in subjects with asthma than in normal controls. The concentrations were also higher in subjects with persistent asthma requiring inhaled steroids or studied within 48 h of an acute severe exacerbation of the condition, than in those with episodic asthma treated with inhaled $beta_2$-agonists only (Pavord et al. 1999).

### *3.3. Aspirin-induced asthma and leukotriene $E_4$ in urine*

Aspirin-induced asthma is a syndrome with a distinct clinical picture, and it affects about 10% of adults with asthma (Szczeklik 1997). Intrinsic asthma has been regarded as a different entity from extrinsic (atopic) asthma, especially if it includes the triad of nasal polyposis, aspirin intolerance, and asthma (Chafee and Settipane 1974, Settipane and Chafee 1977). However, immunological similarities predominate between intrinsic and allergic asthma, and the possibility of local IgE production in the bronchial mucosa in non-allergic asthmatics with normal IgE serum concentrations cannot be excluded (Kroegel et al. 1997). Bochenenek and colleagues (1996) have even found atopy to be related to adverse drug reactions to non-steroidal anti-inflammatory drugs. Irrespective of the definition used, a similar distribution of atopy has been observed in patients with hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAID). Dermographism; chronic urticaria; allergy to antibiotics, metal and food, and high level of IgE have also been shown to be associated with analgesic intolerance in asthmatics (Kalyoncu et al. 1999).

Aspirin-precipited reactions are linked to inhibition of COX (cyclo-oxygenase) which is accompanied by release of cysteinyl leukotrienes (Lee 1993, Szczeklik 1997). Sousa and associates (1997) demonstrated a mean fourfold increase in the percentage of COX-2 (inducible isoenzyme)-expressing mast cells in subjects with aspirin-sensitive asthma. The number of eosinophils expressing COX-2 was increased 2.5-fold in these subjects. $LTC_4$ synthase, the terminal enzyme for cysteinyl leukotrienes, is also markedly overexpressed in eosinophils and mast cells from bronchial biopsy specimens from most patients with AIA (Sampson et al. 1997, Szczeklik and Stevenson 1999). Aspirin may remove $PGE_2$-dependent suppression in all subjects, but only in AIA patients does increased bronchial expression of $LTC_4$ synthase allow marked overproduction of cysteinyl-leukotrienes leading to bronchoconstriction (Cowburn et al. 1998).

Baseline values for urinary $LTE_4$ levels are not different between atopic asthmatics and non-asthmatic individuals (Kumlin et al. 1995), but higher urinary $LTE_4$ levels are reported in aspirin-sensitive as compared to aspirin-tolerant asthmatics and healthy controls (Smith et al. 1992, Sladek and Szczeklik 1993, Kumlin et al. 1995). Bronchial provocation with specific allergen in atopic asthmatics as well as with aspirin in aspirin-intolerant asthmatics is followed by an increase in urinary leukotriene $E_4$, but provocation with histamine does not

provoke release of leukotrienes (Kumlin et al. 1992). In a study by Sladek and Szczeklik (1993), methacholine challenge did not alter urinary $LTE_4$ excretion. No relationship between urinary $LTE_4$ and $PD_{20}$ to histamine was detected by Smith and colleagues (1992).

### *3.4. Effect of anti-asthma drugs on bronchial hyperresponsiveness*

Corticosteroid treatment can reduce the lamina reticularis thickness by modulation of the expression of insulin-like growth factor (IGF)-I, with consequent inhibition of airway infiltration by inflammatory cells, and may therefore also help to prevent remodeling of the airways (Hoshino et al 1998b). However, infiltration of inflammatory cells in the lamina propria of the airways may persist in asthmatic outpatients despite regular treatment with inhaled steroids (Sont et al. 1996), as well as in severe symptomatic asthmatics despite treatment with high-dose glucocorticoids (Wenzel et al. 1997). After discontinuation of inhaled steroid treatment in mild asthma, the $PC_{15}$ value for histamine decreased by an average of 1.5 dose steps in one year, albeit remaining 1.2 steps above the base-line value obtained at the start of the three-year follow up (Haahtela et al.1994).Twelve-month treatment with budesonide in newly diagnosed asthma increased $PD_{20}$ by approximately two doubling dose steps, and during a 6-month follow-up $PD_{20}$ decreased approximately one doubling step (Osterman et al. 1997). Also in childhood asthma under long-term treatment with budesonide, the mean $PD_{20}$ histamine stabilised at 2.1 doubling doses above baseline, but at a subnormal level (van Essen-Zandvliet et al. 1994).

In addition to bronchodilatory effects, $beta_2$-agonists protect against the bronchoconstriction caused by methacholine challenge and measurement of methacholine airway responsiveness can be used in evaluating anti-asthma drugs (Inman et al. 1998, Seppälä et al. 1998). The relative protective dose potency of inhaled $beta_2$-agonists can be determined by comparing their effect on methacholine airway responsiveness (Wong et al. 1998). In a study by Inman and associates (1998), salbutamol (0.2 mg) caused an average shift of 4.11 doubling doses in $PC_{20}$. However, tachyphylaxis to bronchoprotection develops after chronic use of $beta_2$-agonists (Lipworth et al. 1998), and inhaled corticosteroids do not prevent this decrease (Boulet et al. 1998). Regular use of short-acting $beta_2$-agonists increases the late asthmatic reaction to inhaled allergen, in association with an increase in the number of sputum eosinophils and release of ECP (Gauvreau et al. 1997). In asthmatics, theophylline also has a protective activity against methacholine-induced bronchoconstriction (Ferrari et al. 1997, Page et al.1998).

Six weeks´ treatment of patients with aspirin-intolerant asthma (AIA) with the leukotriene-pathway inhibitor zileuton added to existing therapy caused a small but distinct reduction in BHR to histamine and inhibited aspirin-induced bronchoconstriction. Zileuton also inhibited urinary excretion of $LTE_4$ but did not alter airway reactivity to inhaled $LTD_4$ (Dahlen et al. 1998). Zileuton also reduced BAL fluid $LTB_4$ and urinary $LTE_4$ in nocturnal asthmatics, but no significant change was detected in $PC_{20}$ for methacholine (Wenzel et al. 1995). A

single oral dose of zileuton (400mg) was found to increase $PC_{20}$ to histamine by 2.1 doubling doses and the $PD_{20}$ to ultrasonically nebulised distilled water by 1.3 doubling doses (Dekhuijzen et al. 1997). Montelukast (cysteinyl leucotriene receptor antagonist) used for 12 weeks in mild asthma provided significant protection against exercise-induced asthma, but no significant differences in $PC_{20}$ for methacholine were detected (Leff et al. 1998). Four weeks´ treatment with montelukast in chronic adult asthmatics reduced sputum and blood eosinophils, and improved clinical endpoints of asthma (asthma symptoms, $beta_2$-agonist use and morning PEF), but changes in BHR were not reported in the study by Pizzichini´s group (1999). With pranlukast (another cysteinyl leukotriene receptor antagonist) a small but significant reduction (from 0.30 to 0.48 mg/ml) in methacholine responsiveness was observed after a one-week treatment of asthmatic patients (Fujimura et al. 1993b). Pranlukast attenuates allergen-induced early and late responses as well as allergen-induced airway hyperresponsiveness (AHR), which implicates cysLTs as mediators in the AHR seen 24 hours after allergen inhalation (Hamilton et al. 1998).

Inhaled cromolyn sodium protects against aspirin-induced attacks of asthma and also prevents urinary $LTE_4$ excretion in AIA (Yoshida et al. 1998). Compared with placebo, 2 weeks´ treatment with Y-24180 (orally active PAF receptor antagonist) significantly improved the $PC_{20}FEV_1$ value, suggesting that PAF (platelet-activating factor) is an important mediator involved in the BHR of bronchial asthma in humans (Hozawa et al. 1995). Treatment with antihistamines (azelastine for 3 months and ketotifen for 8 weeks) has been shown to be associated with a significant improvement in airway responsiveness to methacholine in atopic asthmatics, this possibly as a result of local bronchial inflammatory cell infiltration (Hoshino and Nakamura 1997a, Hoshino et al. 1997b).

## *3.5. Natural course*

### *3.5.1. The effect of age and maturation*

Airway responsiveness declines with maturation. Normal female children have a greater airway responsiveness to inhaled methacholine than do adults, and this difference is not related to baseline lung size, airway calibre, or delivered methacholine dose (Tepper et al. 1994). In Danish children and adolescents (aged 7 to 17 years at enrolment) examined twice, 6 years apart, the point prevalence of BHR declined from childhood to early adulthood (25% and 6%, respectively), possibly reflecting the increase in airway calibre. The levels of $FEV_1$ and atopy (especially allergy to house dust mite) were important determinants of changes over time in the level of bronchial responsiveness (Ulrik and Backer 1998). In Italy, Forastiere and coworkers (1996) studied a cohort of 7- to 11-yr-old schoolchildren who were restudied after a 3.5-yr interval. An overall decline in the level of BHR was observed paralleling the increase in lung function during this period. The decline was less pronounced in females, and responsiveness was highest in the presence of persistently positive skin prick testing. In children,

increase in nonspecific bronchial responsiveness is also related to the appearance of symptoms during the pollen season, but shows no relationship to the severity of symptoms (Martin-Munoz et al. 1997).

### *3.5.2. The effect of smoking*

Sparrow and associates (1991) studied the influence of age and level of pulmonary function on methacholine responsiveness. A positive methacholine challenge test showed approximately threefold greater odds in association with a 500 ml lower $FEV_1$. Among former smokers a 20-yr increase in age was associated with an approximately fivefold increase in the odds of a positive methacholine challenge test. These findings suggested that methacholine responsiveness increases with advancing age among former smokers even after adjustment for prechallenge level of $FEV_1$. The presence of AHR appears to add approximately 10 ml/year to the decline in $FEV_1$ (24-year follow-up), and AHR was a risk factor for COPD, independent of age and tobacco (Xu et al. 1997). This study was, however, carried out according to the "Dutch hypothesis", which means that the authors included all patients with respiratory symptoms (no distinction made between asthma and COPD)(Vestbo and Prescott 1998).

### *3.5.3. Annual and seasonal changes*

A four-year study by Beckett and colleagues (1997), including healthy, nonasthmatic subjects, showed an annual change in methacholine responsiveness by one or more doubling doses in at least 30% of subjects each year. The within-subject variability in $PD_{20}$ was markedly greater than the corresponding within-subject variability in $FEV_1$. Allergic asthmatic patients have seasonal BHR changes which parallel allergen exposure (van der Heide et al. 1994, Tilles and Bardana 1997) and may be related to specific allergen kinds (Di Lorenzo et al. 1997). In a seven-year follow-up subjects sensitised to laboratory animals showed a minor increase in methacholine responsiveness. During the follow-up, 82% of skin prick test-positive subjects had, however, quitted work involving contact with animals (Sjostedt et al. 1998) The results of the same study support the hypothesis that airway responsiveness in IgE-mediated allergy might start in small airways and subsequently affect large airways.

### *3.5.4. Association with respiratory symptoms*

Subjects who yielded positive methacholine challenge results on initial challenge were found in a study of Muller and group (1994) to be significantly more likely than those with negative results to have nonexertional chest tightness, wheezing and dyspnoea, but not cough. Significant correlations were also found between follow-up (3-10 years) methacholine responsiveness and concurrent symptoms, again with the exception of cough. In the follow-up two thirds of patients continued to have positive or negative methacholine challenge results, and only

one third had a change in status. In a cohort study by Xu and colleagues in Holland (1998) AHR was measured every third year for a 24-year period. Subjects with increased airway responsiveness were more likely than subjects without AHR to develop a variety of respiratory symptoms (chronic cough, chronic sputum expectoration, dyspnoea, asthmatic attacks, persistent wheeze) in any following three-year period (odds ratios 1.4-3.7). Also, they were less likely to report remission of symptoms.

### *3.5.5. Allergic rhinits and bronchial hyperresponsiveness*

Allergic rhinits may represent an intermediate stage in the natural history of BHR in young adults (Dharmage et al. 1998). Allergic rhinitis subjects without evidence of a plateau in methacholine challenge have a degree of diurnal PEF variation similar to that found in patients with mild asthma (Prieto 1998b). Eosinophilic inflammation may be present in subjects with allergic rhinitis and airway hyperresponsiveness even when there are no symptoms of asthma, which could indicate that bronchial eosinophilia is insufficient to cause asthmatic symptoms (Gutierrez et al. 1998). There is an interrelationship between type of allergen, total serum IgE, blood eosinophilic cells and bronchial hyperresponsiveness suggesting that these factors may play a role in the development of bronchial asthma in rhinitis patients (Di Lorenzo et al. 1997).

## *3.6. Clinical significance*

Bronchial hyperresponsiveness relates closely to severity of asthma, frequency of symptoms and the need for treatment (Juniper et al. 1981). In adult asthmatic patients, the number of attacks during a given previous year is the most important background factor related to airway responsiveness on a clinical basis (Tomita et al. 1998). In a study by Balder and colleagues (1998), significant predictors for decreased working capacity in asthmatics were asthma severity, workplace-associated respiratory symptoms and bronchial hyperresponsiveness.

### *3.6.1. Asymptomatic airway hyperresponsiveness*

In a random population sample, hyperresponsive subjects who reported dyspnoea, wheeze or asthma were more likely to show an increase in symptoms expressed by means of the Borg score during histamine provocation than asymptomatic subjects, after adjustment for age, sex, smoking habits, $FEV_1$ and atopy (Brand et al. 1992). These results also suggest that asymptomatic hyperresponders may have variable airway obstruction which is not recognized as breathlessness. Perception of induced dyspnoea also differs between histamine challenge and methacholine challenge (Tetzlaff et al. 1999).

Boulet and associates (1994) studied adult asthmatics with symptomatic remission (no symptoms or medication requirement for at least 2 years). Most “ex-asthmatics“ who considered themselves to be in asthma remission showed a persistent increase in airway responsiveness with or without mild airflow

obstruction, suggesting that reporting symptoms may be an insufficient means of determining degree of remission. In a study by Laprise and Boulet (1997), subjects with asymptomatic AHR showed a greater increase in airway responsiveness and developed asthma symptoms more frequently over a 3-yr period than did normoresponsive subjects. Allergen exposure in sensitised subjects at the time of the study, and genetic predisposition (family history of asthma), seemed to be the main risk factors for the development of symptomatic asthma.

Power and colleagues (1993) obtained bronchial biopsy specimens from clinically healthy subjects with no history of lung disease. Nine of the 27 subjects involved showed bronchial hyperresponsiveness in histamine challenge, and immunohistological analysis showed no evidence of inflammation in subjects both with and without BHR. However, results obtained by Laprise´s group (1999) suggest that asymptomatic airway hyperresponsiveness is associated with airway inflammation and remodelling, and that the emergence of asthma symptoms is associated with an increase in these features. Also in an experimental model, very low repeated doses of allergen have induced significant increased airway reactivity despite lack of evident clinical symptoms or signs of activation of inflammatory cells in peripheral blood (Roquet et al. 1998).

# III AIMS OF THE STUDY

1. To develop and assess a new, rapid, large-dose methacholine challenge test using turbine spirometer for measurements of $FEV_1$ (Studies I ja II).

2. To evaluate the relationship between a rapid methacholine challenge test and different features of physician-diagnosed asthma (Study III).

3. To study the prevalence of asthmatic symptoms and chronic obstructive pulmonary diseases (physician-diagnosed asthma and COPD) in a random adult population sample (Study IV).

4. To study the ability of particular Tuohilampi questionnaire itemss to predict bronchial hyperresponsiveness, defined as a positive reaction to methacholine challenge (Studies IV and V).

5. To study the relationship of bronchial hyperresponsiveness to certain aspects of asthma, especially aspirin intolerance, eosinophilic inflammation markers and smoking (Studies IV and V).

# IV SUBJECTS AND METHODS

## 1. Study populations

A total of 3615 subjects participated in these studies. All subjects and controls were Caucasian. The protocols were approved by the local ethics committee. The main chacteristics of the study populations are given in Table 4.

***Table 4.*** *Characteristics of the study populations*

| | Sex (male/female) | Age (years) |
|---|---|---|
| **Study I** | | |
| Comparison of spirometers (n=42) | 16/26 | 45.5 (18-78)* |
| Repeatability of spirometer measures | | |
| - Healthy subjects (n=10) | 1/9 | 32.0 (18-47)* |
| - COPD patients (n=10) | 7/3 | 60.5 (36-78)* |
| **Study II** | | |
| Hyperreactivive asthmatics (n=11) | 3/8 | 44.1 (19-61)* |
| **Study III** | | |
| Subjects with dyspnoea, wheezing or cough of unknown cause | | |
| - Hyperreactive subjects (n=78) | 28/50 | 44.0 (16.0)† |
| - Non-hyperreactive subjects (n=152) | 62/90 | 44.6 (16.2)† |
| **Study IV** | | |
| A population-based random sample, returned questionnaire (n=3102/ 4300) | 1408/1694 | 42.5 (12.9)† |
| **Study V** | | |
| Aspirin intolerance, pulmonary (n=22) | 7/15 | 47.0 (24-65)* |
| Aspirin intolerance, skin (n=24) | 9/15 | 47.5 (21-66)* |
| Physician-diagnosed asthma (n=39) | 14/25 | 53.0 (20-67)* |
| Asthmatic symptoms, no diagnosis (n=27) | 7/20 | 57.0 (22-67)* |
| Controls (n=19) | 4/15 | 41.0 (25-61)* |

* median (range)
† mean (SD)

The special characteristics of the study subjects are as follows:

**Study I.** Forty-two subjects, 11 healthy volunteers from hospital personnel and 31 patients (16 with obstructive pulmonary diseases, 12 with non-obstructive pulmonary diseases and 3 with various other diseases), attending the Lung Function Laboratory in Helsinki University Hospital were selected for the comparison of the two spirometers. Repeatability of measurements obtained with the pocket spirometer was studied in 20 subjects. Ten of these were healthy subjects from the staff with normal spirometry and no smoking history, and 10 had previously diagnosed COPD according to the criteria of the American Thoracic Society (1987).

**Study II.** Eleven asthmatic patients who had previously exhibited bronchial hyperresponsiveness as determined by a rapid methacholine challenge were recruited for the repeatability study from the outpatient clinic of the Pulmonary Department at Päijät-Häme Central Hospital. The patients had not smoked for at least one year and their total smoking time was less than ten years.

**Study III.** Two hundred and thirty consecutive adult patients tested with the rapid methacholine challenge at the Pulmonary Department of Lahti Central Hospital were studied. The patients were referred to the clinic due to dyspnoea, wheezing, or cough of unknown cause. Patients with previous asthma diagnoses as well as those who had used inhaled steroids during the preceding four weeks were excluded.

**Study IV.** A random sample of 4300 subjects with equal numbers of men and women aged 18-65 years were drawn from the lists of the Finnish Population Register covering the district of Päijät-Häme Central Hospital. The total population of the district is about 208 000 inhabitants, including the city of Lahti. A total of 4300 questionnaires were mailed and 3102 were returned after one reminder, resulting in a response rate of 73%.

**Study V.** The subjects who had returned the questionnaire in study IV were divided into five groups according to history of aspirin intolerance and asthmatic symptoms. One hundred and thirty-one subjects were invited for further studies. The study groups were as follows:

*Group 1.* *Subjects with a history of aspirin intolerance causing shortness of breath or worsening of asthma.*

The population-based sample (3102) included a total of 35 subjects with symptoms consistent with the group definition. A trained nurse interviewed 32 of these subjects and 29 of them were confirmed to have aspirin intolerance. Twenty-two of these participated in the study. Ten subjects (46 %) had physician-diagnosed asthma and 7 of them had been on inhaled corticosteroid treatment.

*Group 2.* *Subjects with a history of aspirin intolerance causing urticaria or angio- oedema but not respiratory symptoms.*
The population-based sample included 50 subjects fulfilling these criteria and 39 of these were interviewed. Thirty-two had a confirmed positive history and 24 took part in the study.

*Group 3.* *Subjects with physician-diagnosed asthma and without a history of aspirin intolerance.*
The population-based sample included 93 subjects in this category. A random sample of 39 subjects participated in the study. Twenty-two of these had been on inhaled steroid treatment.

*Group 4.* *Subjects with a history of symptoms of asthma or attacks of shortness of breath in the past 12 months without asthma or other respiratory diagnosis and no medication and no history of aspirin intolerance.*
The population-based sample included 197 such subjects. A random sample of 27 participated in the study.

*Group 5.* *Subjects without a history of respiratory symptoms and without aspirin intolerance.*
The population-based sample included 1510 such subjects. A random sample of 19 participated in the study.

Twenty-six per cent of all subjects were regular smokers, 43% were irregular smokers or had stopped smoking, and 31% were never-smokers.

## 2. Study designs

**Study I.** $FEV_1$ and FVC as measured with the pocket spirometer were compared with those obtained with the rolling-seal 12 l flow-volume spirometer. At least three maximal expirations were performed with both instruments. Half the measurements were first carried out with the flow-volume spirometer and the other half first with the pocket spirometer. The interval between the measurements with the respective devices was about 10 min for each subject. In the repeatability study each subject performed four consecutive maximal expirations for the measurement of $FEV_1$ and FVC with a single pocket spirometer. The measurements were repeated after 10 min.

**Study II.** The repeatability of a rapid dosimetric method for methacholine challenge was evaluated in 11 asthmatic patients. The methacholine challenges were administered at the same time of day one to seven days apart and the baseline $FEV_1$ was required to be within 10% of the value on the first day. The patients were healthy apart from asthmatic symptoms and were not on any regular medication.

**Study III.** Two hundred and thirty consecutive adult patients tested with the rapid methacholine challenge at the Pulmonary Department of Lahti Central Hospital from May to September 1994 went through the routine tests to exclude chronic asthma. The subjects completed a respiratory questionnaire and carried out 2 week`s twice daily PEF measurement without and with bronchodilator (inhaled 0.2 mg salbutamol). Venous blood samples were collected for measurement of blood eosinophils, spirometry with the bronchodilation test and skin prick tests for common allergens were performed.

**Study IV.** A questionnaire was developed with 37 items based on the Tuohilampi questionnaire (Susitaival and Husman 1996). It also included four new questions concerning aspirin intolerance and nasal polyposis. The questionnaires were mailed in June 1996 with a prepaid return envelope. Those subjects who did not respond within 2 weeks received one reminder letter. After one reminder the overall response rate exceeded 70%, and no further reminders were mailed.

**Study V.** The subjects who had returned the questionnaire in the study IV were divided into five groups according to history of aspirin intolerance and asthmatic symptoms (see Study populations) One hundred and thirty-one subjects completed the further studies. Venous blood samples were collected for measurement of blood eosinophils, serum ECP and MPO. Spirometry with the bronchodilation test and methacholine challenge was performed on different visits. Urine was collected before and 2 hours after methacholine challenge for measurement of $LTE_4$. Skin prick tests for common allergens were also applied.

## 3. Lung function tests

**Study I.** A pocket-size turbine spirometer (Micro Spirometer©, Micro Medical Instruments Ltd, UK) and rolling-seal flow-volume spirometer (CPI 220, cardiopulmonary Instruments, Houston,TX) were used. The subjects were instructed to inspire to total lung capacity and then to exhale with maximal effort through the mouthpiece as rapidly and as far as possible. The nose was closed with a nose-clip. At least three maximal expirations were performed with both instruments and the largest $FEV_1$ and FVC readings were selected for the pocket spirometer.

The turbine volume transducers are preselected by the manufacturer to give volume readings with an accuracy of ±2%. The volume calibration and the linearity of four different pocket spirometers was checked using a calibration pump (Jaeger© 5000 ml piston pump, Erich Jaeger GmbH, Würzburg, Germany). Ten repetitions of fast flow tests (< 1 s) and 10 repetitions of slow flow tests (≥ 6s) were performed.

**Studies III and V.** A bell spirometer with a water seal (Gould 2400©, SensorMedics Corporation, Yorba Linda, CA) was used. The definitions and methods for assessing lung volumes followed the recommendations of the European Coal and Steel Community (Quanjer et al. 1993). Each patient performed a minimum of three blows, and the best of three technically satisfactory forced vital capacities (FVC) and of the first three technically acceptable forced expiratory volumes in one second ($FEV_1$) were reported. The chosen value did not exceed the next highest one by more than 5% or 0.1 l, whichever was best. Measurements of volumes and ventilatory flows were corrected to BTPS (body temperature, pressure, saturated with water vapour). The values are also expressed as percentages of reference (predicted) values for adult Finns (Viljanen et al. 1982).

# 4. Methacholine challenge

An automatic, inhalation–synchronised, dosimetric jet nebuliser (Spira Elektro 2©, Respiratory Care Center, Hämeenlinna, Finland) was used (Nieminen et al. 1987, Nieminen et al. 1988). The nebulisation time was 0.4 second, and nebulisation was set to commence after inhalation of 100 ml. Patients breathed continuously through the mouthpiece and a noseclip was applied with an inspiratory flow rate reaching but not exceeding 0.5 l/s. Using these settings, the mean output of the dosimeter is 6.5±0.3 µl per inspiration (Sovijärvi et al. 1993). The inhalation procedure and $FEV_1$ measurements were practised by each patient prior to the challenge.

After inhalation of 33 µg of saline, methacholine chloride was delivered in 4 successive, increasing doses ranging from 80 µg to a cumulative dose of 6900 µg (Table 5).

***Table 5.*** *Dose protocol for the rapid methacholine test with a Spira Elektro 2 dosimeter: nebulisation time 0.4 s commencing after inhalation of 100 ml of air*

| Dose | Number of inhalations | Methacholine concentration (mg/ml) | Cumulative dose of methacholine (µg) |
|---|---|---|---|
| 1 | 5 | 2.5 | 80 |
| 2 | 5 | 10 | 400 |
| 3 | 5 | 40 | 1700 |
| 4 | 5 | 160 | 6900 |

Because the pH of methacholine solutions is stable, no buffering was needed (Sterk et al. 1993). Inhaled methacholine dose means the amount of agonist delivered to the mouth during inspiration. The $FEV_1$, measured with a turbine spirometer (Micro Spirometer©; Micro Medical Instruments Ltd, Rochester, UK), was used to determine airway response. The volume calibration of the pocket spirometer was checked using 1000 ml (Vitalograph©, Vitalograph, Buckingham, UK), 2000 ml (Jaeger©, Erich Jaeger GmbH, Wüzberg, Germany) and 3000 ml (SensorMedics©, SensorMedics Corporation, Yorba Linda, Ca) calibration pumps. The average values of ten repeated fast injections (<1 s) were 1003 ml, 2026 ml and 2978 ml; the means of proportional errors were 0.9%, 1.3% and 0.7%, respectively.

For the baseline, $FEV_1$ was recorded after inhalation of saline from at least three successive determinations. Ninety seconds after each methacholine dose, three successive determinations of $FEV_1$ were made. Expirations were not continued to the residual volume level in order to minimise bronchoconstriction induced by the expiratory manoeuvre (Sovijärvi et al. 1993). The highest $FEV_1$ values were recorded. The dose interval was 2 minutes. If $FEV_1$ decreased from the baseline ≥20% after any dose, further administration of methacholine was discontinued. After the last methacholine dose, patients inhaled 200 µg of salbutamol aerosol by a metered dose inhaler to resolve bronchoconstriction.

The fall in $FEV_1$ was plotted against methacholine dose on a log scale, and the provocative dose causing a 20% fall in $FEV_1$ ($PD_{20}FEV_1$) was calculated. In Study V the dose-response ratio (DRR) was also calculated for all subjects as the percentage fall in $FEV_1$ at the last dose, divided by total dose administered (O`Connor et al. 1987).

## 5. Questionnaires

**Study III.** Details of a patient's respiratory symptom history were collected by a clinical questionnaire dealing with respiratory symptoms (dyspnoea, wheezing, cough, phlegm production), disposition to atopic diseases, asthma or atopy in first-degree relatives, pets and previous and current treatment (including the use of asthma medication). The patients' smoking histories were also noted.

**Study IV.** The questionnaire was based on the Tuohilampi format, which was created by a group of Finnish researchers from the Finnish Institute of Occupational Health, the National Public Health Institute and several universities for environmental studies of asthma and respiratory disease (Susitaival and Husman 1996). Questions concerning bronchial asthma and related symptoms, atopy and smoking were included. Four new questions concerning aspirin intolerance and nasal polyposis were also added.

The Tuohilampi questionnaire includes items based on several different questionnaires (Medical Research Council (MRC)), 1960, 1966 and 1986;

European Community for Coal and Steel (ECCS) 1987; American Thoracic Society, National Heart, Lung and Blood Institute, Division of Lung Diseases (ATS-DLD-78) 1978; International Union against Tuberculosis and Lung diseases (IUATLD)1986) (Susitaival and Husman 1996). The original international questionnaires have been validated in several studies (Torèn et al. 1993).

## 6. PEF recordings

Patients were carefully instructed by experienced nurses to perform three measurements of maximal peak expiratory flow (PEF) with a mini-Wright peak flow meter every morning and evening for two weeks (Study III). The best value was recorded in the patient's diary. On the second week patients were instructed to perform another three measurements of PEF after inhalation of 0.2 mg salbutamol and record the best value.

## 7. Skin prick tests

Skin prick tests were performed by specially trained nurses with a panel of 12 common allergen extracts (Soluprick©, ALK A/S, Copenhagen, Denmark), with a negative (solvent) and a positive control (histamine dihydrochloride, 10 mg/ml) (Studies III and V). The allergens used were birch, timothy, meadow fescue and mugwort pollen; horse, dog, cat and cow dander; the mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; and spores of the molds *Alternaria alternata* and *Cladosporium herbarum.* The result of the test was considered positive if an allergen caused a wheal size ≥ 3 mm while control solutions gave expected negative and positive results.

## 8. Serum ECP and MPO

Venous blood was collected prior to methacholine provocation into Vacutainer tubes for serum separation, and allowed to clot at 22°C for 60 min (Study V). Serum was separated by centrifugation (1200 x g for 10 min at 22°C) and kept at -70 °C until analysed. Eosinophil cationic protein (ECP) and myeloperoxidase (MPO) were measured by radioimmunoassay using reagents from Pharmacia & Upjohn AB, Uppsala, Sweden.

In studies made at Kabi Pharmacia Diagnostics AB (Uppsala, Sweden), the within-assay coefficient of variation (CV) of ECP at serum level 14 μg/L was 4.3% and the between-assay CV was 8.2%. In a study with 99 apparently healthy adults, the geometric mean was 4.4 μg/L and the 90 and 95 upper percentiles

were 10.1 and 11.3 μg/L. In studies carried out at Pharmacia & Upjohn Diagnostics (Uppsala, Sweden), the within-assay CV of MPO at serum level 379 μg/L was 8.3% and between-assay 9.9%. In a study with 148 apparently healthy adults, the geometric mean was 345 μg/L and the 90 and 95 upper percentiles were 523 and 634 μg/L.

## 9. Urinary $LTE_4$

Urine samples were collected prior to and for the 2 hours following the methacholine provocation, and kept at -70 °C until analysed (Study V). Leukotriene $E_4$ ($LTE_4$) concentrations were measured in serial dilutions by enzyme immunoassay (EIA) using reagents from Cayman Chemicals as described by Kumlin and associates (1995). The results are expressed as ng $LTE_4$ per mmol of creatinine in the urine sample.

The correlation coefficient (r) between added and measured amounts of $LTE_4$ in urine samples was around 0.99. The linearity of the assay was excellent up to added 200 pg/ml, and the precision was also good: the average intra- and interassay variation was 17.6% in the study by Kumlin´s group (1995).

## 10. Study definitions

In Study III, the measurement of bronchial responsiveness was not used as a diagnostic criterion for asthma. Asthma diagnosis was based on a clinical evaluation by the attending chest physicians. The guidelines of the American Thoracic Society (1987) for the diagnosis of bronchial asthma were used. Fulfilment of diagnostic criteria was checked in the analysis phase and the person who classified the patients as asthmatics or nonasthmatics was blinded to $PD_{20}FEV_1$ results. Patients had to have a documented variation in $FEV_1$ or PEF of 15% or greater after medication, or repeatedly (at least two times) a 20% or greater spontaneous daily variation in PEF monitoring during a period of two weeks. In addition, a 15% or greater decrease in $FEV_1$ during an exercise test was a criterion for diagnosing bronchial asthma.

# 11. Statistical analysis

## *Study I*

The individual $FEV_1$ and FVC values obtained with the pocket spirometer were plotted against $FEV_1$ and FVC values measured with the flow-volume spirometer. The linear interdevice correlation coefficient (Pearson`s product movement analysis) was calculated for the parameters. In order to study the agreement between the two spirometers, the individual differences in $FEV_1$ and FVC between the devices were plotted against the average of the measurements. The mean difference represents the bias between the two spirometers. The limits of agreement were determined as two standard deviations from the mean difference (Altman and Bland 1983). These are estimates for the consistency of the bias. The significance of the difference between the two spirometers was evaluated with paired Student`s t-test.

The repeatability of the measurements using a pocket spirometer was assessed in two ways. As the standard deviation of the four readings with each subject was not related to mean, one-way analysis of variance was used to calculate the mean sum of squares (MSW) of the readings within subjects (Armitage 1971). The square root of the sum is an estimate for the standard deviation ($\sigma_e$) of the variation between the readings. The repeatability coefficient (CoR) was calculated using the formula: $CoR=2.83*\sigma_e$. This product gives the maximum difference within which two readings in the same subject lie within a probability of 95% (Altman and Bland 1983). The repeatability coefficient when using the pocket spirometer was compared to that of the flow-volume spirometer in the same study population.

The largest readings of $FEV_1$ and FVC obtained 10 min apart in each subject were compared in order to estimate short-term variation in the measurements when using the pocket spirometer. The mean difference was calculated, and the coefficient of variation representing repeatability was calculated for $FEV_1$ and FVC using one-way analysis of variance and the mean of all the measurements.

## *Study II*

The repeatability of the methacholine challenge was estimated using the methods of Altman and Bland (1983), as recommended by Chinn (1991). Within-subject standard deviation ($SD_W$), i.e. the single determination standard deviation, was calculated. The single determination 95% range, using the formula log $PD_{20}FEV_1 \pm t_{n-1;0.05} \times SD_W$, was used in measuring repeatability. To ensure that within–subject repeatability was not associated with the size of measurements, the difference in log $PD_{20}FEV_1$ was plotted against the mean of log $PD_{20}FEV_1$ and tested using Pearson's correlation analysis. The 95% confidence interval for mean difference was also calculated to establish whether there is an overall change in mean value between the first and second challenge. It was calculated

using the appropriate t–distribution, i.e. the mean difference of log $PD_{20}FEV_1$ $\pm t_{n-1;0.05}$ x SE, where SE is the standard error for mean difference.

### *Study III*

The Chi-square or Fisher's exact tests were used to test differences between proportions and Student's t-test was used to compare group means. The Mann-Whitney t-test was used to compare the groups with respect to the level of bronchial responsiveness, where an arbitrary $PD_{20}FEV_1$ value of 27380 g was given to nonresponders ($PD_{20}FEV_1$ >6900µg). Stepwise logistic regression analysis was performed to investigate the association of age, gender, smoking, number of positive prick results, blood eosinophils, lung function, pets, history of atopy and asthma in relatives, with responsiveness as a dichotomous variable. Methacholine-positive patients ($PD_{20}FEV_1$ ≤6900µg) were defined as hyperresponsive.

Sensitivity (Se), specificity (Sp), and the predictive values of a positive test (PV+) and of a negative test (PV-) for the diagnosis of asthma from the rapid methacholine challenge test—based on the distribution of $PD_{15}FEV_1$ and $PD_{20}FEV_1$ in the clinical material—were calculated according to the following formulas:

$$\text{Se (\%)} = \frac{\text{true-positives}}{\text{true-positives + false-negatives}} \times 100$$

$$\text{Sp (\%)} = \frac{\text{true-negatives}}{\text{true-negatives + false-positives}} \times 100$$

$$\text{PV + (\%)} = \frac{\text{true-positives}}{\text{true-positives + false-positives}} \times 100$$

$$\text{PV - (\%)} = \frac{\text{true-negatives}}{\text{true-negatives + false-negatives.}} \times 100$$

To compare $PD_{15}FEV_1$ and $PD_{20}FEV_1$—as well as to detect the best cut-off point in separating asthmatic and nonasthmatic patients—a receiver operator characteristic curves were graphically constructed by plotting sensitivity against the false-positive rate (1-specificity) for several cut-off point values. The predictive values are strongly influenced by the prevalence of the disease.

With the best cut-off value, the post-test probabilities both of asthma after a positive (PPV) or negative (1-NPV) provocation test result and of non-asthma after a NPV for all possible pre-test probabilities were determined according to Bayes' theorem:

$$\text{PPV (positive predictive value)} = \frac{Pr \times Se}{(Pr \times Se) + (1-Pr) \times (1-Sp)}$$

$$\text{NPV (negative predictive value)} = \frac{(1-Pr) \times Sp}{(1-Pr) \times Sp + Pr \times (1-Se)},$$

where Pr is the prior probability of the disease. The difference between PPV or 1-NPV and the pre-test probability is the positive or negative diagnostic gain of the test. The PPV and 1-NPV for several cut-off points of the methacholine were also calculated (Perpina et al. 1993).

### *Study IV*

The results were analysed with the chi-square test for differences between proportions, and in the case of an ordered explanatory factor, the test of linear trend of proportions was applied (Altman 1991). Relative risks (RR) based on observed prevalences were calculated to compare the patients with physician-diagnosed asthma to subjects without asthma diagnosis. Stepwise logistic regression was used to find the risk indicators of physician-diagnosed asthma. Analysis was made with adjustment for age. Logistic regression analyses were made with a BMDP statistical package (BMDP Software Inc., Los Angeles, Ca).

The prevalences of physician-diagnosed asthma, allergic rhinitis, overall aspirin intolerance and nasal polyposis were established using observed (=crude, non-adjusted) estimates, non-response-adjusted estimates and age-standardised estimates. In adjusting prevalence for non-response the method proposed by Drane (1991) was used. The relative differences between observed and adjusted prevalences were calculated using the formula:

$$\text{Bias (\%)} = 100 \times \frac{\text{(observed prevalence - adjusted prevalence)}}{\text{adjusted prevalence}}$$

Age-standardised prevalences were calculated by the direct method and the European Standard Population was used as the standard (Lillienfeld and Lillienfeld 1980).

### *Study V*

The study population consisted of five separate groups. In statistical analysis groups 1 and 3 were combined to form the asthma group. This combined group was compared to group 4 (asthmatic symptoms without respiratory diagnosis), and to group 5 (controls). In addition group 1 (subjects with aspirin intolerance causing shortness of breath or worsening of asthma) was compared to group 2 (subjects with aspirin intolerance causing urticaria or angio-oedema); and group 4 (asthmatic symptoms without respiratory diagnosis) was compared to group 5 (controls). In pairwise comparisons Fisher's LSD method was used, and in the case of nominal data the Chi-square test was applied. Logarithmic transformations were carried out in the case of skew distributions.

With respect to quantitative inflammatory parameters, t-test for independent samples was used to compare hyperreactive with non-hyperreactive subjects, and smokers with non-smokers. Nominal data were analysed using the Chi-square test. Spearman`s rank correlation coefficient was used to investigate the correlation between change in urinary $LTE_4$ excretion and methacholine dose.

Sensitivity, specificity, and positive predictive and negative predictive values at different cut-off points were calculated to compare serum ECP, MPO and urinary $LTE_4$ as indicators of hyperreactivity. Receiver operator characteristic curves (ROC) for bronchial hyperresponsiveness were constructed by plotting sensitivity against 1–Specificity.

# V RESULTS

## 1. Accuracy and repeatability of the results obtained with a pocket turbine spirometer (Study I)

The linear interdevice correlation coefficient for $FEV_1$ was 0.995 ($p<0.001$) and for FVC 0.935 ($p<0.001$), showing that the measurements were closely related. In the whole material, expressed as ± SD, the pocket spirometer recorded 0.44 l (13% of the mean $FEV_1$) smaller values for $FEV_1$ ($p<0.001$) and 0.64 l (15% of the mean FVC) smaller values for FVC ($p<0.001$) than the rolling-seal spirometer (Study I Table 3). The error was statistically highly significant ($p<0.001$) for both parameters. The magnitude of the difference was proportional to $FEV_1$ ($r=0.812$, $p<0.001$) but not to FVC ($r=-0.159$, NS). The short-term repeatability of the measurements expressed as the coefficient of variation of repeated measurements using the pocket spirometer was 2.2% for $FEV_1$ and 2.3% for FVC.

## 2. Repeatability of the rapid dosimetric method for methacholine challenge using a pocket turbine spirometer for $FEV_1$ measurements (Study II)

The difference in log $PD_{20}FEV_1$ was not related to the average log $PD_{20}FEV_1$ ($r= –0.155$; $p=0.65$). The within-subject standard deviation of log $PD_{20}FEV_1$ was 0.125. Thus, the single determination 95% range was log $PD_{20}FEV_1$ ±0.279, to give ±0.925 doubling doses.

The geometric means of $PD_{20}FEV_1$ (range) were 1792 µg (550–5300 µg) and 1854 µg (450–6900 µg) for the first and second challenge, respectively. The mean difference (SE) between two measurements of log $PD_{20}FEV_1$ was –0.015 (0.056). The 95% confidence interval for the mean difference on the log scale was thus from –0.139 to 0.109 (–0.462 to 0.362 in doubling doses).

## 3. Validation of the rapid dosimetric methacholine challenge test in asthma diagnostics and the risk factors of bronchial hyperresponsiveness (Study III)

The rapid methacholine challenge test was performed with 230 consecutive patients who had dyspnoea, wheezing or a persistent cough of unknown cause. Seventy-eight patients (34%) were methacholine-positive ($PD_{20}FEV_1 \leq 6900$ µg); of these 47 (60%) had a final diagnosis on ATS criteria fulfilling bronchial asthma. The geometric mean provocative dose in methacholine-positive patients was 2013 µg (SD 2010 µg). One hundred and fifty-two patients (66%) were methacholine-negative ($PD_{20}FEV_1 > 6900$ µg), of whom 14 (9%) had bronchial asthma according to clinical evaluation. Increased bronchial responsiveness was strongly associated with the ATS criteria fulfilling asthma ($X^2=68.92$, $p<0.0001$).

The material was consequently divided into two groups of 61 (27%) asthmatic and 169 (73%) non-asthmatic patients. The distribution of $PD_{20}FEV_1$ values in patients with and without asthma is shown in Study III, Figure 1. When $PD_{20}FEV_1$ was used, 47 (77%) of the asthmatics were hyperresponsive (range 40–6900 µg), as were 31 (18%) of the non-asthmatics (range 160–6900 µg). The distribution of $PD_{20}FEV_1$ in 78 hyperresponsive patients is shown in Study III, Figure 2. Using $PD_{15}FEV_1$, 51 (84%) of the asthmatic patients (range 28–6900 µg) and 52 (31%) of the non-asthmatics (range 100–6900 µg) were hyperresponsive. The level of bronchial responsiveness measured by both $PD_{20}FEV_1$ and $PD_{15}FEV_1$ differed significantly between asthmatics and non-asthmatics ($p<0.0001$).

The data on sensitivity, specificity and predictive value of positive and negative results for the diagnosis of asthma from the methacholine challenge test—based on the distribution of $PD_{15}FEV_1$ and $PD_{20}FEV_1$—are shown in Study III, Table 3. The receiver operator characteristic curves of $PD_{15}FEV_1$ and $PD_{20}FEV_1$ for distinguishing asthmatic from nonasthmatic patients are shown in Study III, Figure 3. As may be graphically observed, $PD_{20}FEV_1$ is more discriminative than $PD_{15}FEV_1$ and the best $PD_{20}FEV_1$ cut-off point is 6000 µg (sensitivity and specificity: 0.75 and 0.85, respectively); the difference from 6900 µg is however minimal (sensitivity and specificity: 0.77 and 0.82, respectively). Taking this into account, the post-test probability of asthma or nonasthma with all the pre-test probabilities was determined using a $PD_{20}FEV_1$ cut-off point of 6900 µg (Study III, Figure 4). The best results (PPV: 0.80, NPV: 0.79) were obtained when the pre-test probability was 0.48. The interval security of the test (*i.e.*, the pre-test probability range when PPV and NPV were greater than 50 per cent) was established with a pre-test probability between 0.19 and 0.78. The maximal positive (0.34) and negative (0.31) final gains were achieved when pre-test probabilities were 0.33 and 0.65, respectively (Study III, Figure 5). The curves for PPV and 1-NPV, using several $PD_{20}FEV_1$ cut-off points, were also determined (Study III, Figure 6).

The mean daily variation in PEF was significantly higher in hyperresponsive than in non-hyperresponsive patients (18% vs 10%, p<0.0001). Likewise, there was a significant difference in the number of blood eosinophils between hyperresponsive and non-hyperresponsive patients (306 vs 185 cells/mm3, p< 0.0001; distribution $X^2$=23.90, p<0.00001). Hyperresponsive patients had significantly lower levels of $FEV_1$ and percentage of predicted $FEV_1$ than non-hyperresponsives (2.98 vs 3.23 L/min, p=0.04 and 86 vs 94% p< 0.0001, respectively). There was no association with hyperresponsiveness in FVC and percentage of predicted FVC, nor with respect to age, gender, atopy, history of asthma or atopy in close relatives and keeping pets. Hyperresponsiveness was not significantly more common in the univariate analysis among smoking patients than among non-smokers (43% vs 32%, p=0.18), nor was the difference in levels of hyperresponsiveness significant (p=0.14).

In regression analysis hyperresponsiveness was considered a dichotomous variable using the $PD_{20}FEV_1$ threshold value of 6900 µg. A greater number of positive prick results (OR=1.15, 95% Cl 1.01-1.31), and blood eosinophils (OR=1.004, 95% Cl 1.00-1.01), a lower level of $FEV_1$ (OR=0.56, 95% Cl 0.36-0.87) and current smoking (OR=2.36, 95% Cl 1.00-5.59) were factors significantly associated with an increased probability of hyperresponsiveness. Neither a history of ex-smoking, age, gender, pets nor a history of atopy or asthma in relatives were significantly associated with hyperresponsiveness.

## 4. Prevalence of physician-diagnosed asthma, asthmatic symptoms and COPD in adults aged 18 - 65 years (Study IV)

A total of 4300 questionnaires were mailed and 3102 were returned, yelding in a response rate of 73%. Twenty-five subjects could not be reached at their address, 1166 subjects did not reply and 7 replies had to be excluded from the analyses due to major inaccuracies in the questionnaire responses. Of the responders 1408 (45%) were men and 1694 (55%) were women. The mean age (SD) for men was 43.7 (12.7) years and 43.3 (13.1) years for women. The response was higher among woman (79%) than among men (66%), (p<0.0001); older persons also responded in greater number (80% in 50-65 yrs olds) than younger (67% in 18-34 yrs olds), (p<0.0001). Age had a similar effect in both sexes. Women responded earlier than men (84% of women and 77% of men responded before the reminder, p<0.0001), but late response was not associated with age (p=0.17).

### *Physician-diagnosed asthma, COPD, allergic rhinitis, aspirin intolerance and nasal polyposis*

Observed and non-response-adjusted prevalences of physician-diagnosed asthma, COPD, allergic rhinitis, aspirin intolerance and nasal polyposis are given in Table 6. The corresponding age-standardised prevalences are also given in Table 6.

***Table 6.*** *Prevalences of physician-diagnosed asthma, physician-diagnosed COPD, aspirin intolerance (causing asthma, dyspnoea, urticaria or angio-oedema), nasal polyposis and allergic rhinitis. Observed prevalences (%), non-response-adjusted and age-standardised prevalences (%). Bias (%) in observed versus non-response-adjusted prevalences.*

| | Observed prevalence % (95% Cl) | Non-response-adjusted prevalence % (95% Cl) | Bias % | Age-standardised prevalence* % |
|---|---|---|---|---|
| Asthma | 5.3 (4.6 - 6.1) | 4.4 (3.3 - 5.5) | 21.4 | 5.1 |
| COPD | 3.6 (3.0 - 4.3) | 3.7 (2.7 - 4.8) | 3.7 | 3.3 |
| Aspirin intolerance | 5.8 (5.0 -6.7) | 5.7 (4.4 - 7.1) | 1.6 | 5.7 |
| Nasal polyposis | 4.4 (3.6 - 5.1) | 4.3 (2.8 - 5.8) | 1.4 | 3.9 |
| Allergic rhinitis | 41.6 (39.7 - 43.5) | 37.3 (33.3 - 41.2) | 11.6 | 43.1 |

*European standard population (Lillienfeld and Lillienfeld 1980)

The non-response-adjusted prevalences of physician-diagnosed asthma and COPD in different age groups are given in Study IV, Figure 1 for men and women separately. The observed prevalence of aspirin intolerance causing shortness of breath or attacks of asthma was 1.2% (95% Cl 0.8% to 1.6%). In patients with physician-diagnosed asthma the prevalence was 8.8% (4.4% - 13.2%), and in subjects without asthma diagnosis the prevalence was 0.8% (0.5% - 1.1%). The prevalence of the triad nasal polyposis, aspirin intolerance and asthma was 4.3% in patients with physician-diagnosed asthma (5/7 subjects with the triad also had allergic rhinitis).

### *Asthmatic symptoms*

The observed prevalences of different asthmatic symptoms are given in Table 7. The prevalence of symptoms of asthma or wheezing with shortness of breath during the previous 12 months was 12.8% (12% to 14%). The prevalence of wheeze apart from colds was 13.2% (12 to 14%).

***Table 7. Prevalences of different asthmatic symptoms (95% confidence intervals)***

| | Men (n=1408) | Women (n=1694) | Total (n=3102) |
|---|---|---|---|
| Symptoms of asthma or wheezing with shortness of breath during the previous 12 months | 11.7 (10-13) | 13.7 (12-15) | 12.8 (12-14) |
| -waking at night | 5.6 | 8.1 | 6.9 |
| -worsened at work | 5.1 | 5.3 | 5.2 |
| Wheezing with shortness of breath, total | 18.2 (16-20) | 20.8 (19-23) | 19.6 (18-21) |
| -before school years | 2.4 | 3.0 | 2.7 |
| -during school years | 5.1 | 6.6 | 5.9 |
| -when adult | 15.8 | 18.1 | 17.0 |
| -only with cold | 5.3 | 6.2 | 5.8 |
| -apart from cold | 12.4 | 13.8 | 13.2 |
| -exercise-related | 9.6 | 11.2 | 10.5 |
| -breathing not normal between attacks | 3.7 | 2.2 | 2.9 |
| Cough with wheeze, total | 39.7 (37-42) | 41.6 (39-44) | 40.7 (39-42) |
| -only as a child | 6.6 | 6.5 | 6.6 |
| -only as an adult | 18.8 | 19.4 | 19.1 |
| -as child and adult | 14.5 | 16.4 | 15.5 |
| -only with cold | 23.6 | 26.9 | 25.4 |
| -apart from cold | 15.8 | 15.1 | 15.4 |

BHR+ : $PD_{20}FEV_1 \leq 6900$ µg
BHR- : $PD_{20}FEV_1 > 6900$ µg

## *Risk factors associated with physician-diagnosed asthma*

In the multivariate analysis, the occurrence of physician-diagnosed asthma was explained by aspirin intolerance, allergic rhinitis, nasal polyposis, asthma in close relatives and age. Stepwise logistic regression showed significant effects for aspirin intolerance, allergic rhinitis, nasal polyposis and asthma in close relatives (Study IV, Table 2). The effect of age was not significant, but it was included in the model to show the increased risk in the age group of 50-64 years. Smoking had no independent effect on the occurrence of a diagnosis of asthma.

### *Smoking habits*

More men (30%) than women (18%) were regular, current smokers (Study IV, Table 3), and men had smoked more than women (32% of men and 12% of women had a history of 11 or more pack-years of smoking). The prevalence of COPD increased with pack-years both in patients with and in those without wheeze apart from colds (test for linear trend, $p<0.0001$), but the prevalence of asthma was not affected by smoking (Study IV, Tables 4 and 5). Even symptomatic subjects with a history of >30 pack-years showed only 25.0% prevalence of COPD and 41.7% prevalence of any respiratory diagnosis (Study IV, Table 4).

## 5. Use of serum MPO, ECP and urinary $LTE_4$ in predicting bronchial hyperresponsiviness measured by methacholine challenge (Study V)

### *Comparisons between the groups*

Anthropometric data on the subjects and data on the methacholine challenges and $FEV_1$ measurements are given in Study V, Table 1. There were no hyperreactive subjects ($PD_{20}FEV_1 \leq 6900$ μg) in groups 2 or 5 (subjects without a history of asthmatic symptoms). In subjects with asthma (groups 1 and 3 combined), hyperreactivity (49 %) was observed significantly more often than in group 4 (asthmatic symptoms without diagnosis, 26 %) and group 5 (controls, 0 %), ($p=0.05$ and $p=0.0001$, respectively). The difference between groups 4 and 5 was also significant ($p=0.02$). Among aspirin-intolerant subjects, hyperreactivity was significantly more common in group 1 (respiratory symptoms) than in group 2 (urticaria or angio-edema) (38 % vs. 0 %, $p=0.001$).

In subjects with diagnosed asthma the $FEV_1$ % of predicted value was significantly lower (mean 85 %) than in groups 4 and 5 ($p=0.04$ and $p=0.001$, respectively). The difference between groups 4 and 5 was not significant ($p=0.16$). Among aspirin-intolerant subjects in groups 1 and 2 the difference was not significant ($p=0.67$).

Airway inflammation parameters and skin prick test results are given in Study V, Table 2. The peripheral eosinophil count was higher in diagnosed asthmatics than in controls (0.29 vs. 0.18 cells $mm^{-3}$, $p=0.01$), as was serum ECP (18.6 vs. 12.9 μg/L, $p=0.03$) and serum MPO (337 vs. 266 μg/L, $p=0.06$). The other pairwise comparisons were nonsignificant. In respect of urinary $LTE_4$ and atopic status (skin prick test) there were no differences between the groups.

### *Serum ECP, MPO and urinary $LTE_4$ as indicators of bronchial hyperresponsiveness*

Receiver operator characteristic curves (ROC) for bronchial hyperresponsiveness using different cut-off points are given in Study V, Figure 1. The cut-off points 15 μg/L for ECP, 300 μg/L for MPO and 50 ng/mmol creatinine for $LTE_4$ gave the best sensitivity and specificity. The mean values of serum ECP, MPO and urinary $LTE_4$ in hyperreactive and non-hyperreactive subjects are given in Study V, Table 3.

### *The association between serum ECP and bronchial hyperresponsiveness*

Mean serum ECP was higher in subjects with positive response to methacholine challenge than in those negative to challenge (Study V, Table 3). Mean serum ECP was also increased in current and ex-smokers (means 17.2 (SD 9.7) μg/L and 18.7 (12.1) μg/L), as compared to never-smokers (12.9 (9.0) μg/L, p=0.03 never-smokers vs. current smokers, Study V, Figure 2).

Seventy-two per cent of hyperreactive subjects had serum ECP levels ≥15 μg/L as compared to 35% of non-hyperreactive (p=0.0004, sensitivity 72%, specificity 65%, positive predictive value 43% and negative predictive value 86%). Serum ECP correlated with the blood eosinophil count (r=0.67, p<0.00001). Twenty per cent of those with elevated blood eosinophil count (≥0.4 cells $mm^{-3}$) had, however, serum ECP <15 μg/L and 73% of those with serum ECP ≥15 μg/L had a normal blood eosinophil count (<0.4 cells $mm^{-3}$).

### *Serum MPO and bronchial hyperresponsiveness*

The mean serum MPO was higher in subjects positive to methacholine challenge than in those negative (Study V, Table 3). Mean serum MPO was also higher in current and ex-smokers (means 372 (SD 166) μg/L and 304 (134) μg/L), than in never-smokers (286 (166) μg/L, p=0.04 never-smokers vs. current smokers, Study V, Figure 2).

### *Urinary $LTE_4$ and bronchial hyperresponsiveness*

There were no statistically significant differences between the study groups in baseline urinary $LTE_4$ excretion (Study V, Table 2). During two hours following the methacholine challenge urinary $LTE_4$ excretion increased from 53.8 to 69.0 ng/mmol creatinine in non-hyperreactive subjects, whereas there was no change in $LTE_4$ excretion in hyperreactive subjects (non-hyperreactive vs. hyperreactive, p=0.06). There was no correlation between the increase in $LTE_4$ excretion and methacholine dose (r=0.11, p=0.23).

Aspirin intolerance was also associated with the change in $LTE_4$ excretion after methacholine challenge. In aspirin-intolerant subjects the urinary $LTE_4$ increased from 55.4 to 73.9 ng/mmol creatinine, while in subjects with no history of aspirin

intolerance the change was from 54.5 to 62.5 ng/mmol creatinine (aspirin-intolerant subjects vs. others, p=0.09) No interaction was detected between hyperreactivity and aspirin intolerance (ANOVA, interaction p=0.99). The greatest change in $LTE_4$ was seen in subjects with aspirin intolerance causing urticaria or angio-oedema without respiratory side-effects (from 58.5 to 87.2 ng/mmol creatinine, Study V, Figure 3). The mean increase in these subjects was 28.7 ng/mmol creatinine vs. 7.8 ng/mmol creatinine in others (p=0.02). The lowest increase- if any- was seen in patients with asthma (groups 1 and 3, Study V, Figure 3).

### *History of allergic-like rhinitis or prick test positivity and history of aspirin causing asthmatic symptoms*

In patients with a history of allergic rhinitis, 46.8% had at least one positive prick test reaction compared with 24.1% of patients with no history of allergic rhinitis (Fishers`s exact test p=0.0130). In patients with the history of doctor-diagnosed allergic rhinitis 52.3% had at least one positive prick test result compared with 43.8% of those with self-reported diagnosis (NS, p=0.6165). In patients with a history of aspirin intolerance causing shortness of breath or asthma (Group 1) 17/22 (77%) also had a history of allergic rhinitis. However, only 7/17 (41%) of them, as well as 2/5 (40%) of patients with a negative history of allergic rhinitis and 7/19 (37%) of controls, had at least one positive prick test.

## 6. Validity of particular Tuohilampi questionnaire items as predictors of bronchial hyperresponsiveness (Studies IV and V)

The subsample of persons taking part in Study V was used to test the validity of Tuohilampi questionnaire items on concerning cough with wheeze apart from cold, wheezing with shortness of breath (with breathing normal between attacks) and physician-diagnosed asthma, as predictors of bronchial hyperresponsiveness in terms of sensitivity, specificity, positive predictive value and negative predictive value (Table 8).

Of the subjects examined who had bronchial hyperresponsiveness defined as $PD_{20}FEV_1 \leq 6900$ μg methacholine, 92% had cough with wheeze, 81% reported wheezing with shortness of breath, and 72% reported physician-diagnosed asthma. In this subsample both symptoms and physician-diagnosed asthma showed strong associations with bronchial hyperresponsiveness ($p<0.0001$, $p<0.01$ and $p<0.0001$). In patients with wheeze during the preceding 12 months 44.8% (26/50) were hyperreactive compared with 25.0% (3/12) of patients with no history of wheeze (NS, p=0.3449).

***Table 8.*** *Validity of theTuohilampi questionnaire in respect of questions of a) cough with wheeze apart from cold, b) wheezing with shortness of breath (with breathing normal between attacks) and c) physician-diagnosed asthma as predictors of bronchial hyperresponsiveness, defined as $PD_{20}FEV_1 \leq 6900$ µg methacholine in subsample examined*

| | | | BHR+ | BHR- | TOTAL |
|---|---|---|---|---|---|
| a) Cough with wheeze apart from cold | | Yes | 24 | 25 | 49 |
| | | No | 2 | 45 | 47 |
| | | Total | 26 | 70 | 96 |
| Sensitivity | 92% | (24/26) | | | |
| Specificity | 56% | (45/80) | | | |
| Positive predictive value | 49% | (24/49) | | | |
| Negative predictive value | 96% | (45/47) | | | |
| | | | BHR+ | BHR- | TOTAL |
| b) Wheezing with shortness of breath with breathing normal between attacks | | Yes | 21 | 37 | 58 |
| | | No | 5 | 48 | 53 |
| | | Total | 26 | 85 | 111 |
| Sensitivity | 81% | (21/26) | | | |
| Specificity | 56% | (48/85) | | | |
| Positive predictive value | 36% | (21/58) | | | |
| Negative predictive value | 91% | (48/53) | | | |
| | | | BHR+ | BHR- | TOTAL |
| c) Physician-diagnosed asthma | | Yes | 23 | 17 | 40 |
| | | No | 9 | 71 | 80 |
| | | Total | 32 | 88 | 120 |
| Sensitivity | 72% | (23/32) | | | |
| Specificity | 81% | (71/88) | | | |
| Positive predictive value | 58% | (23/40) | | | |
| Negative predictive value | 89% | (71/80) | | | |

# VI DISCUSSION

## Methodological aspects - Development and assessment of a new, rapid, large-dose methacholine challenge test using turbine spirometer for measurements of $FEV_1$

The pocket turbine spirometer markedly underestimates both $FEV_1$ and FVC compared with the rolling-seal flow-volume spirometer, and cannot be used interchangeably with conventional spirometers. The bias was not consistent for FVC, but for $FEV_1$; after multiplication with a correction factor of 1.15, the mean difference between the two spirometers was low. The discrebancy in $FEV_1$ and FVC between the turbine and rolling-seal spirometer was even more obvious than that previously reported between turbine spirometer and a dry bellows spirometer (Gunawardena et al. 1987, Hosie and Nimmo 1988).

The repeatability of measurements with turbine spirometer is good (the coefficient of variation of repeated measurements was 2.2% for $FEV_1$ and 2.3% for FVC), and it may be used in assessing short-term changes in $FEV_1$ and FVC during provocation tests and in long-term follow-up. The lack of a flow–volume curve does not seem to reduce the repeatability of measurements significantly; however, personnel may have to be more highly trained when assessing the quality of maximal expiration. The variation of a single reading was greater in the COPD group than among healthy volunteers, probably due to differences in subject cooperation and changes in bronchial obstruction among the patients with COPD.

The rapid dosimetric methacholine challenge test performed with a pocket turbine spirometer proved to be as readily reproducible as earlier methods using the Spira Elektro 2 dosimeter and rolling seal spirometer (Nieminen et al. 1988 and Sovijärvi et al. 1993). The single determination standard deviation was low (12.5%); corresponding to a 95% confidence interval of ±0.925 doubling doses compared with ±0.72 doubling doses by the method of Nieminen and associates (1988) and ±0.651 doubling doses in the method of Sovijärvi´s group (1993). The repeatability of our method using the Spira Elektro 2 dosimeter is also highly comparable with those using other dosimeters. The 95% confidence interval, as based on a single determination, is reported to correspond to a ±1.66 fold–difference in $PD_{20}$ from one visit to the next according to the method of Balzano and colleagues (1989), employing the Me.far MB3 dosimeter. In a study by Ryan and associates (1981), using a De Vilbiss 646 nebuliser attached to a Rosenthal–French Dosimeter Model B–2A, the 95% confidence interval for $PC_{20}$, based on a single determination, was approximately the observed value ±1 twofold concentration difference.

The rapid methacholine challenge is clearly less time–consuming than earlier methods employing the Spira Elektro 2. Including bronchodilator aerosol—given

to resolve bronchoconstriction after challenge—the whole test can be completed in 20 minutes. The maximal cumulative methacholine dose is also markedly higher than that described in the previous method (6900 µg vs 2900 µg), making it possible to study the less hyperresponsive end of the unimodal distribution of bronchial hyperresponsiveness to methacholine in asthmatic patients (Nieminen 1992). This is important especially in epidemiological studies and also in clinical practice, e.g. in the follow–up of treatment efficacy.

## Evaluation of the usefulness of the methacholine challenge test in the diagnostics of patients with asthmatic symptoms.

The present rapid method, with its high negative predictive value of 91%, appears at least as efficient as previous dosimetric methacholine tests in the matter of excluding chronic asthma (Nieminen 1992). For a diagnosis of asthma, the predictive value of a positive test is 60%. The sensitivity of the test is, however, related to the characteristics of asthmatic patients and their current status of asthma symptoms (Perpina et al. 1993). In Study III the 230 consecutive adult patients tested were referred to the Pulmonary Department of Lahti Central Hospital due to dyspnoea, wheezing or a cough of unknown cause. Patients with previous asthma diagnoses as well as those who had used inhaled steroids during the preceding four weeks were excluded. The inclusion criteria for our study, as well as the referral pattern of general practitioners, can thus have affected the sensitivity of the test (Knottnerus and Leffers 1992).

The guidelines of the American Thoracic Society (1987) for the diagnosis of bronchial asthma were used in Study III. There is, however, no consensus as to a precise definition of asthma, for which there is no gold standard for diagnosis (Taylor 1997). Twice-daily PEF measurements are regularly used in clinical practice to monitor asthmatic patients and are also considered to be useful devices in screening for asthma in random populations (Boezen et al. 1994). More information is required regarding the use of PEF measurements (Quanjer et al. 1997). It is not known how they should best be applied for their various functions, or whether the diagnostic accuracy increases if measurements are made over longer periods (for example 4 weeks instead of the 2 weeks normally used in clinical practice).

Methacholine and histamine challenges are not suitable for confirming a diagnosis of asthma by reason of their low specificity and low positive predictive value (Rogers and O`Connor 1993, James and Ryan 1997). Although the degree of hyperresponsiveness in asthma is more pronounced than in allergic rhinitis or in chronic bronchitis, a marked overlap prevails (Nieminen 1992). Attempts to impose cut-offs between asthmatics and non-asthmatics, based solely on the degree of nonspecific reactivity, will establish “relative“, but not “absolute“ borders (Townley and Hopp 1988). However, all the patients in Study III who had $PD_{20}FEV_1 \leq 100$ µg also fulfilled the ATS criteria for asthma. This is in concordance with the results previously obtained by Devereux and coworkers

(1996), who found that $PD_{20}$ < 200 µg was practically always associated with active asthma. They also used a high-dose methacholine challenge test (maximal dose 6400 µg).

The shorter protocol seems to yield higher $PD_{20}$ values than those obtained with the previous protocol (Nieminen 1992), as anticipated by Kennedy and Contreras (1993). The Bayesian analysis approach showed that our rapid methacholine challenge is as capable as previous methods of distinguishing between normal and asthmatic subjects (Popa and Singleton 1988, Perpina et al. 1993). A receiver operator curve (ROC) was used to detect the best cut-off point of $PD_{20}FEV_1$ and $PD_{15}FEV_1$ to separate asthmatic from nonasthmatic subjects. $PD_{20}FEV_1$ proved more discriminative; the best $PD_{20}FEV_1$ cut-off point was 6000 µg. The difference from 6900 µg was minimal and further statistics were gathered based on 6900 µg. This can also be argued for inview of the slightly improved ability of the test to determine asthmatics (sensitivity was improved). The best results of the test were obtained when the pre-test probability was 0.48—exactly the result of Perpina´s group (1993). The diagnostic content of the test is maximal when the prior probability of asthma is between 0.33 (maximal positive final gain 0.34) and 0.65 (maximal negative final gain 0.31). Based on the interval security, the rapid test could only be considered if prior probability was established between 0.19 and 0.78. As a common rule with all tests where overlapping occurs between healthy and sick subjects, the rapid methacholine test is useful if not applied to patients with the lowest or highest probabilities of asthma.

One third of the patients referred to a pulmonary outpatient clinic with dyspnoea, wheezing or persistent cough of unknown cause had bronchial hyperresponsiveness (60% of the hyperreactive patients had ATS criteria indicative asthma). Thiadens and colleagues (1998) studied patients with persistent cough who consulted a general practioner; asthma was diagnosed in 39%, COPD in 7% and bronchial hyperrerresponsiveness in 42%. In our study bronchial hyperresponsiveness was unimodally distributed in asthmatic patients, which is in accord with previous results (Cockcroft et al. 1983, Trigg et al. 1990a, Nieminen 1992). Hyperresponsiveness was associated with increased daily variation in PEF and an increased number of blood eosinophils, as well as with decreased levels of $FEV_1$ and percentages of predicted $FEV_1$, positive prick results, and current smoking. A difference was also found between asthmatic and non-asthmatic patients in respect of inflammatory activity in the respiratory tract. These findings are again in accord with previous results (Rijcken et al. 1993, Annema et al. 1995, Ulrik 1995).

## Prevalence of asthmatic symptoms and obstructive pulmonary diseases (physician-diagnosed asthma and COPD) in a random adult population sample.

In Study IV, the response rate was 73%, which is fairly high, but even among those responding a tendency was seen for subjects with a respiratory diagnosis (or symptoms) to be more likely to return the questionnaire earlier than those without a diagnosis (or symptoms) This is in concordance with the results of de Marco´s group (1994) in ECRHS in Italy, where differences in prevalence between early and late responders were almost entirely due to a symptom-related self-selection process: late responders and probably also nonresponders had a lower prevalence than early responders. In order to correct observed prevalence estimates for nonresponse bias, a method proposed by Drane (1991) was used in Study IV. Briefly, a linear trend was assumed in prevalence across subsequent contacts, and the prevalence in nonresponders was extrapolated by fitting a linear regression model to the observed prevalence at each contact (Drane et al. 1991, de Marco et al. 1994).

The prevalences of asthmatic symptoms in Study IV represent the district in southern Finland served by Lahti Central Hospital, and there might be regional differences in Finland as well as in Scandinavia. The results are, however, comparable with those of Björnsson and colleagues (1994) and Pallasaho´s group (1999). The prevalence of breathlessness while wheezing during the previous 12 months was 10.3-12.4% in different areas of Sweden, 13% in Helsinki, and 12.8% in our study. The prevalence of wheezing without cold was 11.4-13.5% in Sweden and 13.2% in our study. The prevalence of allergic rhinitis was higher here (37.3%) than in the Swedish study (21.2-22.2%).

The adjusted prevalence of physician-diagnosed asthma was 4.4%, which is lower than that found in a study in northern Sweden (Lundbäck et al. 1991) with a prevalence of 5.9% of present or past history of asthma, and also lower than in Helsinki study with a prevalence of physician-diagnosed asthma of 6.6% (Pallasaho et al. 1999). The adjusted prevalence of physician-diagnosed asthma in the 25-44 year age group was 3.5 % (95% confidence interval 2.5 to 4.5%), which is lower than the median prevalence of diagnosed asthma in ECRHS, with a prevalence of 4.5% (Burney et al. 1996). The prevalence of COPD in Study IV and that of physician-diagnosed chronic bronchitis in the study by Pallasaho and colleagues (1999) were 3.7%, which is comparable with the 4.1% prevalence of COPD in northern Sweden (Lundbäck et al. 1991). The prevalence of COPD in the present study is clearly too low, as even symptomatic subjects with a history of >30 pack-years had only 25.0% prevalence of diagnosed COPD and 41.7% prevalence of any respiratory diagnosis.

## The validity of particular Tuohilampi questionnaire items as predictors of bronchial hyperresponsiveness.

In Study V, 49% of the patients with asthma, 26% of those with asthmatic symptoms with no diagnosis, and none of the asymptomatic subjects (estimated by the Tuohilampi questionnaire) had bronchial hyperresponsiveness. Asymptomatic bronchial hyperresponsiveness was not detected. A larger cohort of subjects might be needed, but using the Tuohilampi questionnaire it seems in any case possible to discriminate between persons with and without BHR, which is in disagreement with studies with other questionnaires (Kolnaar et al. 1995, Renwick and Conolly 1999).

The importance of asthmatic symptoms has been strongly emphasised recently by Britton and Lewis (1998). In their opinion there is no asthma without symptoms, no point in attempting to recognise an asymptomatic disorder, and no justification for including asymptomatic individuals in the definition of the disease. On the other hand, to avoid underdiagnosed asthma/ COPD, active case finding by asking all patients at risk of pulmonary diseases a few questions about shortness of breath or wheezing is important (den Otter et al. 1998).

## Relationship of bronchial hyperresponsiveness to certain aspects of asthma, especially aspirin intolerance, eosinophilic inflammation markers and smoking.

### *Urine $LTE_4$ and aspirin intolerance*

In the present study measurement of $LTE_4$ in a single sample of urine showed no association with bronchial hyperresponsiveness, which is in agreement with results obtained by Smith and colleagues (1992). As airway hyperresponsiveness was present in ASA-sensitive asthmatics and absent in ASA-sensitive non-asthmatic subjects (urticaria or angio-oedema) in a study of Melillo and associates (1991), BHR was suggested to be a distinctive marker between the two subpopulations of ASA-sensitive patients. Despite the small size of the study groups (18 subjects in the former and 5 in the latter), their results agree with those here (Study V). Croce´s group (1992), however, could detect no significant correlation between $PC_{20}$ to histamine and bronchial challenge with lysine acetylsalicylate (L-ASA) in patients with and without a history of aspirin-induced asthma and control subjects. Acute administration of L-ASA by inhalation protects the asthmatic airways against histamine-induced bronchoconstriction but not against that induced by methacholine (Crimi et al. 1996).

In baseline urine $LTE_4$ excretion no significant differences were found between the study groups. Some previous studies have reported higher urinary $LTE_4$

levels in aspirin-sensitive as compared to aspirin-tolerant asthmatics and healthy controls (Smith et al. 1992, Sladek and Szczeklik 1993, Kumlin et al. 1995). One possible reason for the discrepancy is that in Study V patients were considered aspirin-sensitive according to the history and no provocations were employed. There might also be differences in the ratio of patients with ASA intolerance causing respiratory and skin symptoms. The highest baseline U-$LTE_4$ values in Study V were measured in subjects with a history of ASA intolerance causing urticaria or angio-oedema but not respiratory symptoms (there were, however, no statistically significant differences between the study groups).

The increase in $LTE_4$ after methacholine challenge inhalation also disagrees with previous results (Kumlin et al. 1992, Sladek and Szczeklik 1993, Yoshida et al. 1998). The reason for this is not clear. As the peak increase in urinary $LTE_4$ following bronchial challenge with allergen in atopics or aspirin in aspirin-intolerant asthmatics is fairly short-lived, occurring within 1-3 h after the drop in $FEV_1$ (Kumlin et al. 1995), the pre- and post-challenge urine collection time in our study was chosen to be 2 hours. Kumlin and associates (1995) have found hourly collection of urine to be useful in allergen and aspirin challenges, but this is to minimise the risk of diluting the instaneous releases of leukotrienes. The data in Study V suggest that in non-hyperreactive subjects higher doses of methacholine (6900 μg) provoke extrapulmonary cysteinyl-leukotriene production (possibly in the skin, especially in aspirin-intolerant patients). Inhaled methacholine has been shown to increase $LTB_4$ in asthmatics, but not in healthy controls, without affecting the number of inflammatory cells in BAL fluid. It was impossible to distinguish whether the mediator response in BAL fluid was a direct effect of methacholine, or a consequence of the bronchoconstriction itself (Nowak et al. 1993).

The present data suggesting a negligible role of leukotrienes in bronchial hyperresponsiveness are complicated by the findings of a beneficial clinical action of the 5-lipoxygenase inhibitor zileuton (Dekhuijzen et al. 1997) and a cysteinyl-leukotriene receptor antagonist pranlukast (Fujimura et al. 1993b, Hamilton et al. 1998). These results should, however, be confirmed with a larger number of subjects with treatment given both acutely and long-term (Barnes 1997). Our present data suggest that increased cysteinyl-leukotriene synthesis is not associated with bronchial hyperresponsiveness. Therefore other mechanisms, e.g. increased reactivity to leukotrienes rather than increased leukotriene synthesis, might explain the mediator role of cysteinyl-leukotrienes in bronchial hyperresponsiveness, as would indeed be implied by therapeutic interventions with 5-lipoxygenase inhibitors and leukotriene receptor antagonists.

### *Serum ECP and MPO*

Serum ECP and MPO levels were higher in hyperreactive than in non-hyperreactive subjects, which confirms findings in previous reports (Kristjansson et al. 1994, Carlsen et al. 1997, Nishikawa et al. 1998). However, in a study by Nordman and colleagues (1994) no significant differences in serum MPO values between normoreactive and hyperreactive subjects in methacholine challenge

were found. In asthma and other inflammatory diseases serum ECP is related to the inflammatory activity of the diseases and may be used objectively to adjust and monitor anti-inflammatory treatment in such diseases (Venge et al. 1999). The authors also stress the importance of strict standardisation of blood sampling to ensure reliable and meaningful results.

Serum ECP and MPO levels were higher in current smokers than in never-smokers, suggesting that smoking increases airway inflammation as characterised by eosinophil and neutrophil activation. However, ECP and MPO levels are not changed in smoking asthmatics even with high-dose budesonide (Pedersen et al. 1996) or in COPD patients using inhaled corticosteroids (Keatings et al. 1997b), which would imply that smoking asthmatics and COPD patients might not benefit from these drugs. The results of Study V are also in concordance with those of Jensen´s group (1994), who have shown elevated serum levels of ECP and lactoferrin, another neutrophil marker, in smokers.

A marked overlap in serum ECP levels prevails between adults with asthma and healthy controls, indicating that differences between study groups cannot easily be extrapolated to individuals. The same would be a problem with all markers which reflect only certain aspects of inflammation. Confounding factors such as atopic status (Janson et al. 1997a, Ludviksdottir et al. 1999) and smoking (Jensen et al. 1994, Pedersen et al. 1996) should also be taken into account. In children, evidence is mounting that serum concentrations of ECP and eosinophil-derived neurotoxin (EDN) or eosinophil protein X (EPX) cannot be used to diagnose or monitor childhood asthma (Hoekstra 1999). Pizzichini and associates (1997) showed that the proportion of eosinophils in sputum was a more accurate marker of asthmatic airway inflammation than the proportion of blood eosinophils or serum ECP.

## Clinically significant associations between airway inflammation and bronchial hyperresponsiveness

From a clinical point of view, the main contribution of measurement of non-specific airway responsiveness lies in excluding asthma, by merit of its high sensitivity and high negative predictive value (Rogers and Connor 1993, Antò 1998). Increased BHR is a hallmark of the asthmatic disease process and BHR is closely linked to airway inflammation, but should be regarded as a distinct asthma phenotype (Banik and Holgate 1998). The presence of inflammatory cells is, however, neither sufficient nor necessary for the development of airway hyperresponsiveness (Brusasco et al. 1998).

Stein and associates (1997) have shown that methacholine challenge and PEF variability assessed at age 11, together with markers of atopy, can be used to identify the three different wheezing phenotypes in childhood (transient early wheezing, non-atopic wheezing and IgE-associated wheeze/asthma). Measuring airway responsiveness in COPD is also useful, as it may provide further information on the risk of developing this disease, as well as on the actual and the future condition of this disease (de Jong et al. 1997).

In asthma, bronchial responsiveness testing gives valuable information which is linked both to the patient`s ongoing clinical symptoms and to the pathophysiology of the disease process, i.e. airway inflammation (Banik and Holgate 1998). As improvement in bronchial hyperresponsiveness proved to be with a reduction in the rate of $FEV_1$ loss, Hodgins and colleagues (1998) suggest interventions directed at preventing or reducing nonspecific airway hyperresponsiveness.

The clinical importance of methacholine challenge is one of the main aspects of the present study. Sont and associates (1999b) have recently stressed the value of methacholine challenge as a physiological marker with heterogeneous pathophysiology in monitoring airways inflammation. Reducing BHR in conjunction with optimising symptoms and lung function leads to more effective control of asthma while alleviating chronic airways inflammation (Sont et al. 1999b). It is questionable whether this can be accomplished by including cellular markers of inflammation in asthma management, because the integrative physiological measures rather than specific cells or biochemicals are likely to reflect the complex and chronic inflammatory changes within the airways in this disease (O`Byrne and Postma 1999).

The growing clinical use of long-acting beta-agonists will also increase the need for simple markers of airway inflammation. These drugs, with potential bronchodilating and symptom-relieving effects, can mask increasing inflammation and delay awareness of worsening asthma (McIvor et al. 1998). Until better non-invasive indicators of airway inflammation are identified, all three components of the asthmatic phenotype- airway obstruction, airway hyperresponsiveness and airway inflammation, will need to be assessed both in research and in the clinic (Haley and Drazen 1998).

# VII CONCLUSIONS

1. The rapid dosimetric methacholine challenge test, administered with a pocket turbine spirometer, is as reproducible as previous methods and is less time-consuming than conventional provocations. The pocket turbine spirometer markedly underestimates both $FEV_1$ and FVC compared with the rolling-seal flow-volume spirometer, and cannot be used interchangeably with conventional spirometers. The repeatability of the measurements with pocket turbine spirometer is, however, good, and it may be used in assessing short-term changes of $FEV_1$ and FVC during provocation tests and in long-term follow-up.

2. The rapid dosimetric methacholine challenge test is useful in asthma diagnostics if not applied to patients with lowest or highest probabilities of asthma. The present rapid method, with its high negative predictive value (91%), can be used to exclude chronic asthma. The predictive value of a positive test for ATS criteria-fulfilling asthma is low (60%), and the test is not suitable for confirming a diagnosis of asthma.

One third of the patients referred to the pulmonary outpatient clinic of Päijät-Häme Central Hospital in Lahti with dyspnoea, wheezing or prolonged cough of unknown cause evidence bronchial hyperresponsiveness (60% of the patients with bronchial hyperresponsiveness have ATS criteria-fulfilling asthma). Hyperresponsiveness is associated with increased daily variation in PEF and an increased number of blood eosinophils, as well as with decreased levels of $FEV_1$ and percentages of predicted $FEV_1$, positive prick results and current smoking.

3. The current prevalence of physician-diagnosed asthma among adults in southern Finland (the region with 208 000 inhabitants served by Päijät-Häme Central Hospital) is 4.4% and of diagnosed COPD 3.7%. However, the prevalence of asthmatic symptoms in adults exceeds 10%, and there is obvious under-diagnosis especially of COPD. Allergic rhinitis, nasal polyposis and aspirin intolerance are associated with an increased risk of asthma.

4. In a population-based subsample half of the patients with asthma, one fourth of subjects with asthmatic symptoms with no diagnosis, and none of the asymptomatic subjects (estimated by Tuohilampi questionnaire) have bronchial hyperresponsiveness. Using Tuohilampi questions concerning cough with wheeze apart from cold and wheezing with shortness of breath (with breathing normal between attacks) it is possible to rule out persons without bronchial hyperresponsiveness.

5. Elevated serum ECP and MPO, but not urinary $LTE_4$ (even in subjects with a history of aspirin intolerance), predict bronchial hyperresponsiveness to methacholine. However, a considerable overlap in serum ECP and MPO levels prevails between subjects with and without bronchial hyperresponsiveness. The subject`s smoking history must also be taken into account when serum ECP and MPO are considered.

Baseline urine $LTE_4$ is not elevated in patients with a history of aspirin intolerance, but urine $LTE_4$ is increased after methacholine challenge especially in non-hyperreactive patients with aspirin intolerance causing urticaria or angio-oedema. These findings are not in concordance with previous results and should be confirmed in further studies.

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Jouni Hedman

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# X APPENDIX

# An English translation of the questions used in the Study IV.

1. Have you ever had a cough with wheezing?
   **0** no (*skip to question 4*) **1** yes
2. Has this cough appeared
   **2** only when child **3** only in adult life **4** both when child and in adult life
3. Has this cough appeared
   **2** only at times when you had a chest infection (for example flu or bronchitis)
   **3** also during times when you did not have chest infection
4. Have you at any time had attacks of shortness of breath with wheezing *(Attacks here mean occasional shortness of breath, not for example normal breathlessness after exercise)*
   **0** no **1** yes
5. Do you have a physician-diagnosed asthma?
   **0** no **1** yes
   *if you have never had attacks of shortness of breath with wheezing, nor physician-diagnosed asthma, skip to question 13.*
6. Has your breathing been normal between the attacks of shortness of breath
   **0** no **1** yes
7. Have you had symptoms of asthma or attacks of shortness of breath with wheezing
   **2** only at times when you had a chest infection (for example cold or bronchitis)
   **3** also during times when you did not have a chest infection
8. Have you had symptoms of asthma or attacks of shortness of breath with wheezing during or soon after physical strain?
   **0** no **1** yes
9. Have you had symptoms of asthma or attacks of shortness of breath with wheezing *(choose all proper alternatives)*
   **2** before school age **3** at school age **4** in adult life
10. In the past 12 months, have you had symptoms of asthma or attacks of shortness of breath with wheezing?
    **0** no *(skip to question 13)* **1** yes
11. In the past 12 months, have you been woken at night by symptoms of asthma or an attack of shortness of breath with wheezing?
    **0** no **1** yes
12. Do symptoms of asthma or shortness of breath get worse at work?
    **0** no **1** yes
13. Have you ever had hay fever or other allergic rhinitis connected with for example pollens or animals?
    **0** no **1** yes **9** don´t know *(skip to question 15)*
14. Is the allergic rhinitis mentioned in the question 13 diagnosed by a doctor?
    **0** no **1** yes **9** don´t know
15. Have you had hypersensitivity to painkillers (for example Aspirin)
    **0** no *(skip to question 17)* **1** yes **9** don´t know *(skip to question 17)*

16. Is the hypersensitivity to painkillers manifested as *(choose all the proper altervatives)*

**2** shortness of breath **4** eczema
**3** worsening of asthma **5** other symptom, what?_____

17. Has there been found polyps in your nose?

**0** no **1** yes **9** don´t know

17. Does any of your sisters, brothers or parents have asthma now or have had asthma before?

**0** no **1** yes **9** don´t know

19. Have you ever been smoking?

**0** no *(skip to question 24)* **1** yes

20. Have you ever been smoking <u>regularly</u> (almost every day at least one year)?

**0** no *(skip to question 22)* **1** yes

21. How many years have you been smoking in all *(take off the periods of unsmoking, which have lasted more than 6 months)*

years

22. How much on average do you smoke now or smoked before you stopped smoking?

**2** cigarettes **3** pipe **4** cigars

23. Do you smoke nowadays?

**0** not at all **2** yes regularly **3** yes occasionally

24. Has a doctor diagnosed any of the following diseases in you?

*(choose all the proper alternatives)*

**2.** chronic bronchitis
**3** emphysema
**4** bronchiectasis
**5** asthma
**6** not any of these

# KYSELY HENGITYSTIEOIREISTA

1. Onko Teillä koskaan ollut yskää, johon on liittynyt hengityksen vinkumista?
**0** ei (*siirtykää kysymykseen 4*) **1** kyllä
2. Onko tällaista yskää ollut
**2** vain lapsena **3** vain aikuisena **4** sekä lapsena että aikuisena
3. Onko tällaistä yskää ollut
**2** vain hengitystietulehdusten (esim. flunssan tai euhkoputkentulehduksen) yhteydessä
**3** muulloinkin kuin hengitystietulehdusten yhteydessä
4. Onko teillä koskaan ollut hengenahdistuskohtauksia, joihin on liittynyt hengityksen vinkumista (*henhenahdistukohtauksella tässä tarkoitetaan ajoittaista hengenahdistusta, mikä ei ole tavallista hengästymistä*)
**0** ei **1** kyllä
5. Onko lääkäri todennut teillä astman?
**0** ei **1** kyllä

*Jos Teillä ei ole ollut hengenahdistuskohtauksia, joihin liittyy hengityksen vinkumista eikä Teillä ole lääkärin toteamaa astmaa, siirtykää kysymykseen 18.*

6. Onko hengityksenne ollut hengenahdistuskohtausten välillä normaalia?
**0** ei **1** kyllä
7. Onko astmaoireita tai hengenahdistuskohtauksia, joihin on liittynyt hengityksen vinkumista, ollut
**2** vain hengitystietulehdusten (esim. flunssan tai keuhkoputkentulehduksen) yhteydessä
**3** muuloinkin kuin hengitystietulehdusten yhteydessä
8. Onko astmaoireita tai hengenahdistuskohtauksia, joihin on liittynyt hengityksen vinkumista, ollut ruumiillisen rasituksen aikana tai pian sen jälkeen?
**0** ei **1** kyllä
9. Onko astmaoireita tai hengenahdistuskohtauksia, joihin on liittynyt hengityksen vinkumista ollut (*valitkaa kaikki sopivat vaihtoehdot)*
**2** ennen kouluikää **3** kouluiässä **4** aikuisena
10. Onko astmaoireita tai hengenahdistuskohtauksia ollut
**2** ympärivuotisena **3** vain kausiluonteisesti
(*siirtykää kysymykseen 12*)
11. Milloin kausittain esiintyviä astmaoireita tai hengenahdistuskohtauksia on yleensä ollut?
*(valitse kaikki sopivat vaihtoehdot)*
**2** keväisin **3** kesäisin **4** syksyisin **5** talvisin **6** ei liity vuodenaikoihin
12. Onko teillä ollut astmaoireita tai hengenahdistuskohtauksia, joihin on liittynyt hengityksen vinkumista, viimeisten 12 kuukauden aikana ?
**0** ei (*siirtykää kysymykseen 16*) **1** kyllä

13. Miten usein astmaoireita tai hengenahdistuskohtauksia on ollut viimeisten 12 kuukauden aikana **JA** viimeiseen 12 kuukauteen sisältyvän oirekauden aikana ? (esim. siitepölykauden aikana)

| | |
|---|---|
| **2** päivittäin tai lähes päivittäin | **6** päivittäin tai lähes päivittäin |
| **3** viikottain | **7** viikottain |
| **4** kuukausittain | **8** vain silloin tällöin |
| **5** harvemmin | |

14. Oletteko joutunut heräämään yöllä astmaoireiden tai vinkuvan hengenahdistuskohtauksen takia viimeisten 12 kuukauden aikana?

**0** ei **1** kyllä

15. Pahenevatko astma- tai hengenahdistusoireet työssä ollessa?

**0** ei **1** kyllä

16. Valitkaa seuraavasta luettelosta ne tekijät, joiden olette itse todennut pahentavan tai aiheuttavan astmaanne tai vinkuvia hengenahdistuskohtauksia *(valitkaa kaikki sopivat vaihtoehdot)*

| | |
|---|---|
| **2** siitepölyt | **11** lämmin ilma |
| **3** kotieläimet | **12** kylmä ilma |
| **4** huonepöly | **13** kuiva ilma |
| **5** muu pöly | **14** kostea ilma |
| **6** tupakansavu | **15** ruumiillinen rasitus |
| **7** tuoksut tai hajut | **16** henkinen rasitus |
| **8** käryt, kaasut | **17** rasitus kylmässä |
| **9** pakokaasut | **18** särkylääkkeet ( aspiriini tai muut särkylääkkeet) |
| **10** pesu- tai puhdistusaineet | **19** joku muu mikä?_____ |

17. Oletteko käyttänyt astmalääkkeitä viimeisten 12 kuukauden aikana ?

**0** en

kyllä lääkkeiden nimet

**2** vain tarvittaessa, _____

**3** säännöllisesti, _____

18. Onko Teillä koskaan ollut heinänuhaa tai muuta esimerkiksi siitepölyihin tai eläimiin liittyvää allergista nuhaa?

**0** ei (*siirtykää kysymykseen 20*) **1** kyllä

**9** en tiedä (*siirtykää kysymykseen 20)*

19. Onko kysymyksessä 18 mainittu allerginen nuha lääkärin toteama?

**0** ei **1** kyllä **9** en tiedä

20. Onko Teillä esiintynyt särkylääkeyliherkkyyttä (esim. aspiriinille)

**0** ei (*siirtykää kysymykseen 22*) **1** kyllä **9** en tiedä (*siirtykää kysymykseen 22)*

21. Ilmeneekö yliherkkyys särkylääkkeille *(valitkaa kaikki sopivat vaihtoehdot)*

| | |
|---|---|
| **2** hengenahdistuksena | **4** ihottumana |
| **3** astman pahenemisena | **5** muuna oireena,minä?_____ |

22. Onko Teillä todettu nenässä polyyppeja?

**0** ei **1** kyllä **9** en tiedä

23. Onko Teiltä poistettu nenästä polyyppeja?

**0** ei **1** kyllä

24. Onko jollakin sisaruksistanne tai vanhemmistanne nyt tai aiemmin ollut astmaa ?

**0** ei **1** kyllä **9** en tiedä

25. Onko jollakin sisaruksistanne tai vanhemmistanne nyt tai aiemmin ollut allergista nuhaa, allergista silmätulehdusta tai allergista ihottumaa?

**0** ei **1** kyllä **9** en tiedä

26. Mikä oli lasten lukumäärä lapsuudenkodissanne (itsenne mukaanluettuna)?

27. Monesko lapsi perheessä itse olitte?

28. Oletteko koskaan tupakoinut?

**0** en (*siirtykää kysymykseen 35*) **1** kyllä

29. Oletteko koskaan tupakoinut säännöllisesti ( lähes joka päivä ainakin yhden vuoden ajan)?

**0** en (*siirtykää kysymykseen 32)* **1** kyllä

30. Minkä ikäinen olitte aloittaessanne säännöllisen tupakoinnin?

31. Kuinka monta vuotta olette tupakoineet yhteensä? (*vähentäkää tupakointivuosista yli 6 kk kestäneet tupakoimattomuusjaksot*)

32. Kuinka paljon poltatte nykyisin tai poltitte ennen lopettamista keskimäärin vuorokaudessa?

**2** savukkeita **3** piippua **4** sikareita

33. Tupakoitteko nykyisin?

**0** en lainkaan **2** kyllä säännöllisesti **3** kyllä satunnaisesti

34. Koska olette tupakoinut viimeksi?

**2** eilen tai tänään
**3** 2 päivää-1 kuukausi sitten
**4** yli 1 kuukautta - ½ vuotta sitten
**5** yli ½ vuotta - vuosi sitten
**6** yli vuosi sitten, **minä vuonna?** vuonna 19 _____

35. Tupakoiko joku säännöllisesti kotonanne sisätiloissa, kun olitte lapsi?
*(valitkaa kaikki sopivat vaihtoehdot)*

**0** ei **2** kyllä, äiti **3** kyllä, isä **4** kyllä, joku muu

36 Kuinka monta tuntia keskimäärin olette nykyisin päivittäin sisätiloissa, joissa tupakoidaan?

**0** tuskin yhtään tuntia

37. Onko lääkäri todennut Teillä jonkin seuraavista hengityselinsairauksista?
*(valitkaa kaikki sopivat vaihtoehdot)*

**2** krooninen keuhkoputkentulehdus eli krooninen bronkiitti
**3** keuhkonlaajentuma eli keuhkoemfyseema
**4** keuhkoputkien laajentumat eli bronkiektasiat
**5** astma
**6** ei mitään näistä

# ORIGINAL PUBLICATIONS